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Substance use is a significant public health concern due to association with a host of negative psychosocial outcomes. Understanding etiology of substance use remains a key research priority. The current study aimed at contributing to the extant literature of etiology of substance use by examining gene–gene interaction and gene–environment interaction effects in predicting trajectories of substance use from early adolescence to young adulthood, as well as considering gender differences in substance use trajectories and genetic and gene–environment interaction effects. Using data from the National Longitudinal Study of Adolescent Health ($N = 13,749$), this study examined trajectories of alcohol, cigarette, and marijuana use from age 13 to age 32 and evaluated how genes (i.e., DRD4 and 5-HTTLPR) and parenting quality independently and interactively predicted individual's likelihood of following different trajectories of substance use over time. Growth mixture modeling analyses identified distinct trajectories of alcohol, cigarette, and marijuana use from early adolescence to young adulthood. Results from multinomial logistic regression analyses provided evidence for gene–gene interaction and gene–environment interaction effects as well as gender differences in these effects in predicting substance use trajectories. Results highlighted the importance of considering the heterogeneity of substance use, examining multiple genes (and environmental factors) in combination, and considering gender differences in understanding etiology of substance use.

PREDICTING SUBSTANCE USE TRAJECTORIES FROM EARLY ADOLESCENCE
TO YOUNG ADULthood: EXAMINATION OF GENE–GENE INTERACTION,
GENE–ENVIRONMENT INTERACTION AND GENDER DIFFERENCES

by

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Substance use has long been a public health concern as it has been shown to be associated with a host of negative psychosocial outcomes such as mental health problems, risky sexual behaviors, drug abuse and dependence. Adolescence has been known as the period in which substance use normatively begins as national estimates of high school students in 2012 showed that about 51.5% of adolescents have ever drunk alcohol, 28.7% smoked cigarettes, 31.0% used marijuana, and 16.1% used illicit drugs such as inhalant, cocaine, and heroin (Johnston, O'Malley, Bachman, & Schulenberg, 2012). By young adulthood, about 57.5% of American youth are current drinkers (among whom 39.5% engaged in binge drinking in the past month), 38.1% are current smokers, 18.7% currently use marijuana and 21.3% currently use illicit drugs including marijuana (Substance Abuse and Mental Health Services Administration, 2013). Although most young people reduce their substance use as they make transitions into adulthood, long-term substance use trajectories vary (e.g., White, Pandina, & Chen, 2002). For example, while many individuals never use drugs or alcohol, others' substance use may occur only during a developmentally-limited period like adolescence. Other individuals may initiate substance use early in adolescence and continue to use substances in adulthood (life-course persistent use) and have elevated risk for seriously adverse outcomes such as substance abuse, dependence, and internalizing disorders (Chassin, Pitts, & Prost, 2002;

Flory, Lynam, Milich, Leukefeld, & Clayton, 2004). Other individuals may begin using substances only in young adulthood (adult-onset use) after abstaining during adolescence. To the extent that these different trajectories of substance use may be differentially associated with later outcomes in life (e.g., addiction, mental health, physical health outcomes), identifying trajectories of substance use behaviors that individuals follow from adolescence to adulthood and identifying those who are at risk for continuation of substance use has significant public health value.

A wealth of research has been conducted to examine the etiology of substance use, with an increasing focus on understanding genetic contributions while considering environmental influences. It is now well acknowledged that substance use is a multifactorial phenotype that is influenced by the interplay of genes and environments. A review of the behavioral genetic studies on substance use (Hopfer, Crowley, & Hewitt, 2003) suggested that genetic effects explained a significant proportion of variance in substance use, although the proportion of variance attributed to genetic effects differed to some extent depending on the specific substance under consideration, necessitating examination of use of different substances separately. Recent molecular genetic studies on substance use have started to identify specific genes that are linked to substance use. For example, the dopamine receptor genes (e.g., DRD4) and the serotonin transporter gene (i.e., 5-HTTLPR) have been shown to be associated with risk of substance use. Specifically, 7-repeat allele of DRD4 has been found to be associated with higher risk for heavy drinking (Laucht, Becker, Blomeyer, & Schmidt, 2007), and smoking (Laucht, Becker, EI-Faddagh, Hohm, & Schmidt, 2005) among adolescents and problematic

alcohol use among college students (Ray et al., 2008). Adolescents with a short allele of 5-HTTLPR have been shown to be more likely to use alcohol, tobacco, and illicit drugs compared to individuals who do not have a short allele of 5-HTTLPR (Kaufman et al., 2007; Merenäkk et al., 2011). However, findings were not consistent as some studies failed to find an association between DRD4, 5-HTTLPR and substance use (e.g., Creemers et al., 2011; Dick, Plunkett, et al., 2007). These inconsistent findings from molecular genetic studies might be the result of differences in sample characteristics and measurement of substance use across studies, and may also point to the potential importance of considering moderators of genetic effects.

Although research has started to accumulate in documenting the influence of DRD4 and 5-HTTLPR on substance use behaviors, there are major gaps in the extant literature. First, it is still largely unknown whether DRD4 and 5-HTTLPR interact in influencing substance use. This is important as researchers have suggested that gene–gene interactions are ubiquitous and failing to consider interacting effects among genes may lead to inconsistent findings regarding main effects of genes (Moore, 2003; Moore & Williams, 2009; Sillanpää & Auranen, 2004). If there are significant interactions between DRD4 and 5-HTTLPR, it would suggest that effects of these two genes can only be understood in combination and that previous studies considering single genes (i.e., DRD4, 5-HTTLPR) separately might have yielded incomplete or misleading results. Second, it is still relatively unknown whether genetic effects on substance use are universal or vary across individuals. As such, research is needed to examine moderating factors for genetic effects on substance use to understand the context under which and for

whom genetic effects are most influential for substance use. For example, it may be that genetic risk for substance use is particularly salient for individuals who experience stressful life events or adverse home environments such as poor parenting (Covault et al., 2007; Dick, Viken, et al., 2007).

Parenting might be an important moderating factor to be considered for genetic effects on substance use. Prior research has demonstrated the importance of positive parenting behaviors in protecting substance use among adolescents. Parenting behaviors, such as parental monitoring, parental warmth and support, and parental involvement have been found to be associated with lower substance use both in adolescence and young adulthood (Barnes, Reifman, Farrell, & Dintcheff, 2000; Bogenschneider, Wu, Raffaelli, & Tsay, 1998; Broman et al., 2006; Pilgrim, Schulenberg, O'Malley, Bachman, & Johnston, 2006). Recently, there has been evidence suggesting interaction effects between genes and parenting in relation to substance use. For example, using a twin-study design, Dick and colleagues (Dick, Viken, et al., 2007) found that genetic effects on adolescent substance use were greater for adolescents who experienced lower levels of parental monitoring. Another study of African American adolescents suggested that the association between 5-HTTLPR and adolescent substance use was stronger among adolescents experiencing lower levels of supportive parenting (Brody et al., 2009). These studies suggest that parenting plays an important role in qualifying genetic effects in relation to substance use.

Gender is another important factor to be considered as a potential moderator for genetic effects on substance use. Recent national estimates suggest that there is no

gender difference in alcohol and cigarette use among adolescents; however, among older individuals, males have higher rates of alcohol use, binge drinking and cigarette use than females. Among individuals aged 12 or older, males are more likely than females to be current users of illicit drugs including marijuana (Substance Abuse and Mental Health Services Administration, 2013). Despite gender differences in overall rates of substance use, prior research suggested that trajectories of substance use are similar across gender (Flory et al., 2004); however, generally there is a greater proportion of males in the trajectory of heavy use than females (e.g., Chassin et al., 2002; Chassin, Flora, & King, 2004). Although evidence is still relatively rare, it has been suggested that there are gender differences in genetic effects on individuals' psychosocial outcomes related to substance use. For example, using data from the National Longitudinal Study of Adolescent Health (Add Health), 5-HTTLPR was found to be associated with risk for antisocial behavior among females but not males (Li & Lee, 2010). DRD4 has been found to be associated with delinquency and thrill seeking among adolescent males but not females (Dmitrieva, Chen, Greenberger, Ogunseitan, & Ding, 2011) and impulsivity among adult males but not females (Reiner & Spangler, 2011). As such, although not yet examined, it is possible that gender moderates influences of DRD4 and 5-HTTLPR on substance use trajectories. In addition to potential gender differences in genetic effects, there might also be gender differences in gene–environment interaction effects in relation to psychosocial outcomes including substance use. Although rarely examined, there has been some evidence of three-way interaction between gender, gene, and environment. For example, interactions between 5-HTTLPR and childhood maltreatment have been

found among females but not males in predicting antisocial behavior (Li & Lee, 2010) and criminal behavior and substance use (Vaske, Newsome, & Wright, 2012) among adolescents from Add Health.

This study aimed to fill the gaps in the extant literature by examining gene–gene and gene–environment interactions in predicting substance use trajectories while considering gender differences. Specifically, the current study examined 1) whether DRD4 and 5-HTTLPR independently and interactively linked to substance use trajectories from adolescence to young adulthood, 2) whether parenting interacted with DRD4 and 5-HTTLPR in predicting trajectories of substance use, and 3) whether there were gender differences in genetic and gene–environment interaction effects in linking to substance use trajectories.

CHAPTER II

THEORETICAL FRAMEWORKS: GENE–ENVIRONMENT INTERPLAY AND DEVELOPMENT

Many developmental theories have emphasized the importance of considering person–context interaction, and more specifically gene–environment interplay, in understanding human development. The conceptualization of gene–environment interaction in understanding substance use trajectories in this study was informed by two theoretical frameworks: the transactional model developed by Sameroff (2009), and the probabilistic–epigenetic framework proposed by Gottlieb (1998, 2000a). Both of these theoretical frameworks emphasize the importance of considering interactions between genotypes and environments in understanding development over time.

The Transactional Model

The transactional model (Sameroff, 2009) posits that development of any process in the individual is influenced by its interplay with processes in the individual’s context over time. Individual development is considered to occur through continuous dynamic interactions of the developing individual and the experiences he or she acquires from the environment. The individual and environment are conceptualized to be interdependent and reciprocally influencing each other over time.

An important concept in the transactional model is regulation. According to Sameroff (2009), individual development is conceptualized to involve co-regulation

between the self (i.e., self-regulation) and the environment (i.e., other-regulation). On one hand, individuals' self-regulatory capacities for development can only function if there is a social environment that is engaged in "other-regulation" to provide the social emotional and cognitive experiences that need to be self-regulated; on the other hand, regulations from the social settings to enhance development will not be effective if individuals' self-regulatory capabilities are too compromised. As such, according to this perspective, environmental influences on development are dependent on individuals' characteristics (e.g., genetic predispositions); likewise, influences of individuals' characteristics such as genotypes on development are also moderated by environmental experiences.

The transactional model emphasizes the roles of biological regulations in development, particularly the roles of genotypes. Specifically, this model is depicted by having genotype, phenotype and environment reciprocally influencing each other over time, with phenotype being placed in the middle of the model (Sameroff, 2009, Figure 1.3, pp. 15). It is conceptualized that individuals' behavior at any time point is a product of the transactions between the phenotype (i.e., observable characteristics or behaviors of the individual), the environment (i.e., individual's external experience with the environment), and the genotype (i.e., individual's biological organization) over time. According to this model, while genotypes influence individuals' phenotypic characteristics, genetic activities are also influenced by characteristics of the individual (i.e., phenotype), particularly the epigenome (i.e., a network of chemical compounds surrounding DNA that modifies the function of genome without altering DNA sequences)

that is influenced by both internal and external environments. Likewise, while environmental experiences (e.g., parenting) influence individuals' phenotypes, characteristics of the individual also play active roles in changing the environment. For example, children with different temperaments may stimulate different parenting behaviors from their parents, which in turn influence children's behaviors.

The Probabilistic–Epigenetic Framework

Gottlieb's probabilistic–epigenetic framework, also referred to as probabilistic epigenesis, takes a developmental systems view and posits that development occurs through bidirectional influences within and between hierarchical levels of influences (i.e., genetic activity, neural activity, behavior, and environment) (Gottlieb, 1991, 1998, 2000a, 2007). It is conceptualized that all parts of the developmental system are capable of influencing all of the other parts of the system, however indirectly that influence may occur. Specifically, genes are conceptualized as an integral part of the developmental system and genetic activities (e.g., gene expression) are conceptualized to be affected by influences from other levels of the system, including influences from both the internal and external environments of the individual. Genes require environmental and behavioral inputs to function appropriately during the normal course of individual development, and thus genetic activities should be viewed within a holistic developmental–physiological framework (Gottlieb, 2000a).

The probabilistic–epigenetic framework questions what Gottlieb referred to as the central dogma of molecular biology, which holds that information flows in only one direction from the genes to the structure of the proteins to structural maturation and to

individual functions and activities, and that influence of genes on development is unidirectional and predetermining. On the contrary, this framework posits that the information flow is bidirectional, and that genes express themselves appropriately only in responding to internally and externally generated stimulations. Normally occurring environmental influences (e.g., parenting), as well as behavioral and neural activities are conceptualized to affect gene activity, with environmental influences on genetic activities generally, not always, being mediated by neural and behavioral activities (Gottlieb, 1998, 2000a). Thus, genetic influences on development are suggested to be probabilistic rather than predetermined (Gottlieb, 1998, 2000a, 2000b, 2007).

The proposition that genetic effects are probabilistic also challenges the idea of genetically determined reaction range, which is the idea that genotypes limit the possible number of phenotypes to a few rather than many and that there are upper and lower bounds for phenotypes as restricted by genotypes that cannot be transcended (Gottlieb, 2007). But this proposition is in line with the conceptualization of the norm of reaction in biology that takes into account gene–environment interactions that are considered to be ubiquitous in human development (Gottlieb, 2007). The norm of reaction utilizes a developmental systems point of view and holds that phenotypic outcomes are non-predictive as each new environment is expected to have a different influence on developmental outcomes given the same genotypes (Gottlieb, 2007; Platt & Sanislow, 1988, as cited in Gottlieb, 1991). As such, individuals who have the same genotypes will not necessarily have the same neural and behavioral outcomes, depending on their environmental experiences (Gottlieb, 2007), that is, gene–environment interactions are

expected such that genetic effects on developmental outcomes over time are moderated by environmental factors.

Taken together, both the transactional model and the probabilistic–epigenetic framework recognize the bidirectional or reciprocal nature of the relationship between the individual and environment and emphasize the importance of gene–environment interaction in the course of development over time. These theoretical frameworks inform the current study in conceptualization of understanding gene–environment interaction in the development of substance use trajectories over time, and more specifically, the moderating roles of parenting and gender on the effects of two specific genotypes: DRD4 and 5-HTTLPR. According to these theoretical perspectives, parenting can be considered as a normally occurring external environment that contextualizes or moderates genetic effects through dynamic interactions between parenting and the individual’s genotypes. Gender can also be considered as a moderator of genetic effects as it potentially links to both internal (sex-related biological environment, e.g., hormones) and external (gender-relevant social environment, e.g., socialization, gender roles) environments that are conceptualized to modify genetic effects.

CHAPTER III

LITERATURE REVIEW

Although rates of substance use during both adolescence and adulthood have generally decreased over the past few decades, it is still relatively prevalent among adolescents and young adults (Johnston et al., 2012). Substance use has been well demonstrated to be associated with various adverse outcomes such as accidents, risky behaviors, substance abuse and dependence (e.g., Marshall, 2014). As such, substance use remains a significant public health concern. Although prior research has identified a number of risk and protective factors related to substance use, ranging from individual characteristics to social environments, there is a need for research that moves beyond environmental risk/protective factors only to also consider genetic contributions in understanding etiology of substance use. The current study extended the extant literature by focusing on examining substance use trajectories and genetic and gene–environment interaction effects in relation to substance use trajectories. More specifically, this study focused on independent and interactive effects of DRD4 and 5-HTTLPR, and the moderating roles of parenting and gender in relation to substance use trajectories from adolescence to young adulthood. The following sections reviewed the literature relevant for the key constructs and associations focused in the current study.

Trajectories of Substance Use

National statistics demonstrate the continuing widespread use of cigarette, alcohol, and marijuana by individuals in their adolescence and young adulthood (Johnston et al., 2012). For each of these substances, longitudinal studies have demonstrated that overall substance use increases from early to late adolescence, generally peaks during emerging adulthood (ages 18 to 25) and declines thereafter (Chen & Jacobson, 2012). However, not all individuals follow this general pattern of substance use over time. For many individuals, onset of substance use occurs during early adolescence, whereas some individuals start substance use in late adolescence or young adulthood. For many individuals, use of substances peaks during emerging adulthood and then they “mature” out of substance involvement upon successfully navigating developmental transitions and responsibilities associated with career and family, whereas others continue on a trajectory of frequent substance use (e.g., Tucker et al., 2005; Wichers, Gillespie, & Kendler, 2013). These different trajectories of substance use might have different implications for individuals’ long-term well-being. For example, individuals who continue to use substances beyond adolescence or emerging adulthood might be at greater risk for long-term physical and psychosocial problems (Brook, Lee, Finch, & Brook, 2014; Chassin et al., 2002).

While substance use researchers have increasingly recognized the heterogeneity in developmental courses of substance use among individuals, only recently have data analytic methods advanced to foster rapid growth in research on developmental trajectories of substance use within a heterogeneous sample. Recent longitudinal studies

have identified distinct trajectories of alcohol (Chassin et al., 2002; Flory et al., 2004; Jackson & Sher, 2005; Tucker, Ellickson, Orlando, Martino, & Klein, 2005; Wichers et al., 2013), cigarette (Brook et al., 2008; Chassin, Presson, Pitts, & Sherman, 2000; Tucker et al., 2005; White, Pandina, & Chen, 2002), and marijuana use (Brook et al., 2014; Kandel & Chen, 2000; Schulenberg et al., 2005; Windle & Wiesner, 2004). Overall, for each of these substances, some prototypical trajectories have been identified, including (but not limited to) a non-user/low use trajectory, a chronic/life-course persistent heavy use trajectory, a developmentally-limited, declining/maturing out trajectory, and a late-onset, increasing trajectory. This is consistent with Moffitt's (1993) conceptualization of differential developmental courses of antisocial behavior (i.e., adolescence-limited versus life-course persistent) and it is argued that etiological processes for different developmental trajectories of antisocial behavior might be different (Moffitt, 1993). Antisocial behaviors that are developmentally/adolescence-limited may be predominantly influenced by environmental factors such as parenting or peer associations; whereas antisocial behaviors that are life-course persistent or chronic are more heavily influenced by individual characteristics such as genetic predispositions (Moffitt, 1993).

Although increasing research efforts have attempted to identify distinct developmental trajectories of substance use using longitudinal design, many of these longitudinal studies used regional samples that were not nationally representative, and thus raising questions regarding the generalizability of findings (e.g., Chassin, et al., 2004; Tucker et al., 2005). Of the longitudinal studies that used nationally representative samples, most have focused on change in substance use within a limited developmental

period, such as from early adolescence to late adolescence (Duncan, Duncan, & Strycker, 2006; Jackson, Sher, Cooper, & Wood, 2002), and from late adolescence to young adulthood (Jackson et al., 2008; Muthen & Muthen, 2000). As such, longitudinal studies using nationally representative samples to examine trajectories of substance use from early adolescence to young adulthood are lacking. Moreover, studies that examine the interplay of genetic and environmental factors in influencing trajectories of substance use from early adolescence to young adulthood are scarce. As such, it remains largely unknown how genetic and environmental factors independently and interactively influence individual's likelihood of following different trajectories of substance use from early adolescence to young adulthood.

DRD4, 5-HTTLPR and Substance Use

Molecular genetic studies have made great advances in identifying genes related to substance use, including genes that involve in the metabolism of drugs and genes that involve in neurotransmitter systems that are related to substance use behaviors. In particular, genes that are involved in the dopamine or serotonin neurotransmitter systems have attracted a lot of research attention, as these neurotransmitter systems have been suggested to be involved in substance use behaviors (Goodman, 2008).

DRD4. The dopamine system has been known to be involved in behavioral activation, motivation, and reward processing (Ikemoto & Panksepp, 1999). The dopamine D4 receptor gene (i.e., DRD4) encodes the D4 subtype of dopamine receptor. This gene maps to 11p15.5, contains a 48 bp Variable Number Tandem Repeat (VNTR) polymorphism in the third exon (van Tol et al., 1992), which results in ten allelic

products comprised of from 2-11 repeat units (see the method section for more details about this gene). The longer variants (≥ 7 -repeat) appear to blunt the intracellular response to dopamine in vitro as compared with other variants (Asghari et al., 1995), and thus individuals who carry the longer allele of DRD4 have been suggested to be at higher risk for “reward deficiency syndrome” (Blum, Cull, Braverman, Chen, & Comings, 1997) and risky behaviors including substance use and abuse. These individuals, with blunted response to dopamine, are believed to not feel as much reward and pleasure from everyday activities, thus they tend to seek out reward by engaging in more rewarding/stimulating activities such as risky behaviors including substance use.

A number of studies have examined association between the DRD4 gene and substance use behaviors in both community and clinical samples. Individuals who carry the long (i.e., ≥ 7 -repeat) allele of DRD4 are suggested to be at higher risk for substance use and abuse, although some studies failed to find an association between DRD4 and substance use (see McGeary, 2009 for a review). Using a diverse, non-clinical sample of adolescents from Add Health, Vaske and colleagues found that 7-repeat allele of DRD4 was associated with higher risk of marijuana use and comorbid depression (Bobadilla, Vaske, & Asberg, 2013), as well as higher risk for following a trajectory of increasing marijuana use from adolescence to young adulthood (Vaske, Boisvert, Wright, & Beaver, 2013). Laucht and colleagues found that 7-repeat allele of DRD4 was associated with higher levels of alcohol use (Laucht et al., 2007) and higher rates of lifetime smoking and poorer quit rates for smoking (Laucht et al., 2005) in a sample of high risk adolescents. However, other studies failed to find a significant association between DRD4 and

substance use. For example, DRD4 genotype was not associated with regular alcohol use and marijuana use in a sample of Dutch adolescents from the general population (Creemers et al., 2011). DRD4 genotype was also not associated with quantity of alcohol consumed among adolescents and young adults in a genetic subsample of Add Health (Hopfer et al., 2005). In a sample of psychiatric inpatients, DRD4 was also not associated with adolescent alcohol and other drug misuse (McGeary, Esposito-Smythers, Spirito, & Monti, 2007). As such, previous studies have yielded mixed findings regarding associations between DRD4 and substance use.

5-HTTLPR. The serotonin neurotransmitter system in the brain has been suggested to be involved in various functions such as mood, affect, and behavioral inhibition (e.g., Daw, Kakade, & Dayan, 2002). The serotonin transporter (5-HTT) is central to the serotonergic system as it regulates the magnitude and duration of serotonergic response and serotonin levels by the reuptake of serotonin from the synaptic cleft. Transcriptional activity of the serotonin transporter gene is modulated by a polymorphism in the 5-HTT gene-linked polymorphic region (5-HTTLPR) located in the 5' regulatory region of the gene (Heils et al., 1996). A deletion/insertion in the 5-HTTLPR creates a short allele and a long allele, the former leading to lower transcriptional activity and thus lower expression of the serotonin transporter gene (Heils et al., 1996; Lesch et al., 1996). Thus, individuals who carry the short allele of 5-HTTLPR might have dysfunction of serotonin transporter, which has been implicated in several psychiatric disorders (e.g., substance abuse) and problem behaviors such as aggression (Feinn, Nellissery, Kranzler, 2005; Seo, Patrick, & Kennealy, 2008).

Previous studies have demonstrated an association between 5-HTTLPR and substance use behaviors. Specifically, the presence of the short allele variant of 5-HTTLPR has been suggested as a risk factor for substance use among adolescents and adults, although findings are mixed (see Feinn et al., 2005 for a review). For example, among a sample of African American adolescents, 5-HTTLPR short allele was found to be linked with increase in substance use over time (Brody et al., 2009). 5-HTTLPR short allele was also associated with increase in alcohol use among a sample of maltreated adolescents (Kaufman et al., 2007). Among a sample of adolescents in Europe, 5-HTTLPR short allele was also found to be associated with more substance use, although the effects were stronger for older adolescents than for younger adolescents, and stronger for tobacco use than for alcohol use (Merenäkk et al., 2011). Similarly, Vaske et al. (2012) found that 5-HTTLPR short allele was associated with more alcohol use problems and higher frequency of marijuana use in a sample of young adults in Add Health. On the contrary, some studies found that 5-HTTLPR short allele was protective against substance use. For example, Olsson et al. (2005) found that 5-HTTLPR short allele was associated with lower binge drinking during adolescence and young adulthood in an Australian sample representative of the broad adolescent population. In a study of college students, it was found that men with the homozygous long allele 5-HTTLPR genotype reported higher relief drinking (i.e., drinking to cope) than did men with the homozygous short allele 5-HTTLPR genotype (Armeli, Conner, Covault, Tennen, & Kranzler, 2008). Other studies failed to find a significant association between 5-HTTLPR and substance use. For example, 5-HTTLPR was found to be not associated

with alcohol dependence in the Collaborative Study on the Genetics of Alcoholism sample (Dick, Plunkett, et al., 2007).

Understanding Mixed Findings of Genetic Effects

One explanation for inconsistent findings regarding genetic effects on substance use is that a specific genotype, such as 7-repeat allele of DRD4 and short allele of 5-HTTLPR, in and of itself does not guarantee risk for substance use, as other genetic and social–environmental factors also play important roles (Hopfer et al., 2003; Stallings et al., 2005). As such, these mixed findings across studies to some extent point to the importance of examining potential moderators of genetic effects in relation to substance use. Social–environmental factors, particularly parenting and gender, are promising moderators for genetic effects, as albeit still relatively rare, there has been evidence suggesting that effects of genes such as DRD4 and 5-HTTLPR on substance use varied as a function of parenting behaviors (e.g., Beach, Brody, Lei, & Philibert, 2010; Olsson et al., 2013; Otten, Barker, Hulsink, & Engels, 2012; Vaske et al., 2013) and across gender (Armeli et al., 2008; Brody et al., 2014; Merenäkk et al., 2011).

That the majority of studies examining genetic effects on substance use considered independent effects of each gene, rather than considering genetic effects in the context of other genes, might have also contributed to the mixed findings. Researchers have suggested that epistasis (i.e., gene–gene interaction) is ubiquitous (Moore, 2003; Moore & Williams, 2009) and might serve as potential reasons for non-replication of findings among studies that considered effects of single genes (Sillanpää & Auranen, 2004). In addition, power to detect effects of a single gene is likely to be reduced if

epistasis is present and as such consideration of gene–gene interaction has been recommended (Cordell, 2002). As such, it is important to consider potential interactive effects of DRD4 and 5-HTTLPR when examining the influence of DRD4 and 5-HTTLPR on substance use behaviors.

In fact, it has been suggested that there is functional interaction between the serotonin system and the dopamine system. More specifically, serotonin is suggested to have a regulatory control over dopamine, meaning that a disruption of the serotonin system will lead to a disruption of the dopamine system and affect dopamine–mediated behaviors (Kapur & Remington, 1996; Wong, Feng, & Teo, 1995). It has also been suggested that the serotonergic system functions as opponent to the dopaminergic system, and that an imbalance between the two systems may confer risk for development (Daw et al., 2002). Thus, it is plausible that genes involving in the two systems (e.g., DRD4 and 5-HTTLPR) will function interactively in relation to phenotypes such as substance use. There has been some empirical evidence demonstrating interactions between DRD4 and 5-HTTLPR in relation to substance use and related outcomes, although the nature of interaction is still unclear. For example, 5-HTTLPR short allele was found to be associated with more externalizing problems only among adolescents who were carriers of 7-repeat allele of DRD4, whereas no association was found between 5-HTTLPR and externalizing behaviors among adolescents who did not carry 7-repeat allele of DRD4 (Hohmann et al., 2009). In another study of adolescents, interaction between DRD4 and 5-HTTLPR was found for substance use among girls but not boys. Specifically, the long

allele of 5-HTTLPR was associated with higher substance use among girls only when the 7-repeat allele of DRD4 was not present (Skowronek et al., 2006).

The inconsistency in research findings regarding effects of genes on substance use as reviewed above might have also in part resulted from different sample characteristics. Many of these studies used at-risk samples (e.g., maltreated adolescents), and it is possible that etiological processes to substance use are different between these individuals and individuals in the general population. It should be noted that many of the studies reviewed here were of relatively small sample size that included either a selective at-risk sample or a regional community sample of adolescents or young adults. As such, it is unknown whether extant findings on associations between genes and substance use hold for the general population of adolescents and young adults in the United States. Moreover, previous studies with small sample size might have been underpowered in detecting significant genetic effects.

It is noteworthy that previous research examining associations between genotypes and substance use has mostly adopted a cross-sectional design. The few studies that employed a longitudinal design has mostly focused on examining genetic effects on rates of change in substance use over time or whether genetic effects on substance use vary across age, using analytic strategies such as latent growth curve analysis (e.g., van der Zwaluw et al., 2010) and repeated measures analysis of variance (e.g., Merenäkk et al., 2011). Prior research has rarely examined the role of genotypes in differentiating trajectories of substance use over time. One exception is the study by Vaske and colleagues (Vaske et al., 2013) in which they examined the association between DRD4

and trajectories of marijuana use using the first three waves of data from a genetic subsample of Add Health. They found that 7-repeat allele of DRD4 was associated with membership in the late-onset increasing trajectory and the chronic trajectory of marijuana use, whereas DRD4 was not associated with membership in the desister (i.e., engaged some marijuana use during early adolescence and declined over time) trajectory, relative to the non-user trajectory. These findings suggested that genetic effects on substance use trajectories might vary depending on nature of the trajectory. That is, genetic effects might be more relevant for substance use trajectories commonly considered to be more problematic, such as the chronic or persistent heavy use trajectory. These findings were consistent with Moffit's (1993) proposition that influence of individual characteristics such as genetic predispositions are more salient for antisocial behaviors that are life-course persistent or chronic than for antisocial behaviors that are developmentally/adolescence-limited which may be more heavily influenced by environmental factors.

Utilizing longitudinal designs to examine trajectories of substance use from early adolescence to adulthood in studies of genetic effects on substance use is important, as researchers have suggested that genetic influence may vary over the course of substance use (Kendler, Schmitt, Aggen, & Prescott, 2008). More specifically, twin studies have suggested that genetic factors have little influence on the initiation of substance use in childhood and early adolescence which is influenced primarily by environmental influences, whereas establishment of substance use patterns is more strongly influenced by genetic factors (Hopfer et al., 2003; Kendler et al., 2008). In a longitudinal study of

adolescents, short allele of 5-HTTLPR was found to be associated with greater increase in alcohol use over time but was not associated with initial level of alcohol use among adolescents (van der Zwaluw et al., 2010). As such, it is possible that the mixed findings in previous studies regarding effects of DRD4 and 5-HTTLPR on substance use, in part, was a result of differences in participants' stages in their courses of substance use development, as well as differences in phenotypic measurement of substance use outcomes (e.g., onset of substance use versus frequency or quantity of use). Thus, by using a longitudinal design that captures both initiation and patterns of substance use from early adolescence to young adulthood, we could have a better understanding about how genotypes such as DRD4 and 5-HTTLPR relate to substance use, as well as how genotypes may interact with environmental factors in influencing substance use over time.

Gene–Environment Interaction: the Role of Parenting

Parenting is an important environmental factor for adolescent development, and parenting during adolescence has been demonstrated to have long-term effects for individuals' well-being in adulthood (e.g., Aquilino & Supple, 2001). Specifically in reference to substance use, researchers often view family as a prosocial primary socialization unit that influences adolescents' substance use with the focus on positive aspects of parenting behaviors and parent–child relationships that reduce risk for substance use (Oetting & Donnermeyer, 1998). Parents, as primary socialization source, have direct effects on adolescent's behaviors such as substance use through socialization processes. These processes involve creating close connections with the adolescent, direct communication of norms toward substance use, and direct monitoring, encouragement,

and sanction of substance use norms and behaviors (Oetting & Donnermeyer, 1998). For example, parents might influence their adolescents' substance use through maintaining a warm, involved relationship with adolescents, conveying their attitudes toward substance use, and monitoring adolescents' behaviors. Adolescents who maintain a close relationship with their parents, experience high levels of parental support and monitoring, and have parents promoting norms against substance use, might be less likely to associate with deviant peers, have less opportunity to engage in substance use, and have disapproving attitudes against substance use, and thus may be less likely to engage in substance use behaviors. Supporting this line of reasoning, parenting behaviors such as parental support, parental monitoring, and parental involvement have been associated with lower substance use among adolescents, both concurrently and prospectively (Bahr, Hoffmann, & Yang, 2005; Barnes et al., 2000; Bogenschneider et al., 1998; Bronman et al., 2006; Clark, Shamblen, Ringwalt, & Hanley, 2012; Fletcher, Steinberg, & Williams-Wheeler, 2004; Pilgrim et al., 2006). In addition to influencing adolescent substance use, parenting behaviors during adolescence have also been linked to substance use trajectories from adolescence to adulthood. For example, in a community sample of 481 individuals, those who reported having poor relationship quality with their parents during adolescence were more likely to follow an early-onset trajectory of marijuana use from early adolescence to young adulthood, relative to following a non-use trajectory (Flory et al., 2004).

Sameroff's (2009) transactional model posit that individual's biological predispositions (e.g., genes) transact with contextual processes (e.g., parenting) to

influence development over time. Such transactions are called gene–environment interactions. Gene–environment interactions occur when genetic variation alters an individual’s sensitivity to specific environmental effects or when environmental factors exert differential control over genetic effects (Kendler & Eaves, 1986). That is, gene–environment interactions can occur in two forms: genetic effects moderated by environmental factors and/or environmental effects moderated by genetic predispositions.

Different conceptual perspectives regarding the nature of gene–environment interaction effects have been proposed in the literature. The two perspectives that are most popularly discussed are the diathesis-stress (i.e., dual risk) model (Rende & Plomin, 1992) and the differential susceptibility hypothesis (Belsky & Pluess, 2009). The diathesis-stress model posits that, some individuals, because of their specific “vulnerability”, are disproportionately or even exclusively likely to be affected adversely by environmental stress. This “vulnerability” can be behavioral (e.g., difficult temperament), physiological or endophenotypic (e.g., heightened biological reactivity to stress), or genetic (e.g., 5-HTTLPR short allele). According to this perspective, individuals carrying certain “vulnerability genes” or “risk alleles” are most likely to suffer developmental problems such as substance use problems, when they are exposed to environmental adversity. It is noteworthy that the diathesis-stress model emphasizes individual vulnerability to adverse environments and pays little attention to how individuals might respond differentially also to positive environments. Implicit to this perspective is the assumption that individuals who differ in vulnerability develop differently principally under the condition of environmental stress, whereas individuals

respond similarly to supportive or enriched environments (Ellis, Boyce, Belsky, Bakermans-Kranenburg, & Van IJzendoorn, 2011).

Different from the diathesis-stress model, the differential susceptibility hypothesis, instead of viewing that individuals differ in their “vulnerability” to environmental adversity, perceives that individuals differ in their “susceptibility” to environmental influences, including influences from both adverse and supportive environments. That is, some individuals with “susceptibility” predispositions (e.g., behavioral, physiological, neurobiological, genetic) are more adversely affected by disadvantaged environments but also benefit more from advantaged environments (Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007). Differential susceptibility is demonstrated when the interaction reflects a crossover pattern that covers both the positive and negative aspects of the environment (Belsky & Pluess, 2009; Roisman et al., 2012). According to the differential susceptibility hypothesis, individuals with certain genetic susceptibility alleles (e.g., 5-HTTLPR short allele) are more likely to have developmental problems (e.g., substance use) when they experience adverse environment (e.g., low parental support), but are less likely to have developmental problems when they experience supportive environments (e.g., high parental support), compared to those who do not have the genetic susceptibility alleles.

Parenting might function as a protective factor that attenuates negative effects of genetic risk on substance use via interaction with genes, in addition to positive effects of parenting in reducing risk for substance use. On the other hand, influence of parenting behaviors on individual’s substance use might be moderated by individual’s genotype. In

fact, there has been increasing evidence of interaction effects between genes and parenting in relation to psychosocial outcomes including substance use, although findings have been mixed.

Some previous studies found significant gene by environment interaction effects supporting the diathesis-stress model. For example, using a representative sample of young Australians from the Victorian Adolescent Health Cohort study, Olsson et al. (2011) found that 7-repeat allele of DRD4 was associated with higher tobacco, marijuana, and alcohol use (binging), and that significant interaction between DRD4 and insecure attachment was found for problematic tobacco and marijuana use (not for binge drinking) such that those who carried the 7-repeat allele of DRD4 along with insecure attachment had highest level of tobacco and marijuana use. In another study, the negative effect of having a short allele of 5-HTTLPR on adolescent substance use was shown to be stronger when supportive parenting was lower among a sample of African American adolescents (Brody et al., 2009).

Other studies point to gene by environment effects in support of the differential susceptibility hypothesis. For example, in a longitudinal study of 381 Dutch adolescents, Otten et al. (2012) found a significant interaction effect between parental monitoring and DRD4, such that when experiencing low levels of parental monitoring, adolescents who carried the 7-repeat allele of DRD4 reported higher levels of and greater increase in marijuana use over time; whereas when experiencing high levels of parental monitoring, those who carried the 7-repeat allele of DRD4 were less likely to use marijuana and showed smaller increase in frequency of marijuana use, compared to individuals without

the 7-repeat allele. Beach and colleagues (Beach et al., 2010) found that the Strong African American Families (SAAF) parenting intervention program was protective for adolescents with the 7-repeat allele of DRD4 (not for those with 4-repeat allele of DRD4). Specifically, among adolescents with the 7-repeat allele of DRD4, those who were in the control group (presumably had lower positive parenting) increased in substance use over time, whereas those who were in the intervention group (experienced higher positive parenting given the effectiveness of the intervention program) experienced a slight decrease in substance use. There has not been evidence of 5-HTTLPR by parenting interaction in relation to substance use supporting the differential susceptibility hypothesis. However, differential susceptibility of the 5-HTTLPR short allele has been demonstrated in interaction with parenting and negative life events for other psychosocial outcomes such as neuroticism (Pluess, Belsky, Way, & Taylor, 2010) and positive affect among adolescents (Hankin et al., 2011).

Yet, other studies failed to find significant interactions between DRD4, 5-HTTLPR, and parenting in relation to substance use. For example, in a sample of 1192 Dutch adolescents representing the general population, DRD4 was found to be not associated with regular alcohol and marijuana use and did not interact with parenting (i.e., rejection, overprotection, and emotional warmth) in predicting alcohol and marijuana use (Creemers et al., 2011). Similar to the inconsistent findings regarding main effects of DRD4 and 5-HTTLPR on substance use, the mixed findings regarding interaction effects between these genes and parenting in relation to substance use might in part due to differential sample characteristics across studies and differences in

measures of parenting and substance use outcomes. It is also possible that gene by parenting interaction effects might vary across individuals, for example, as a function of gender.

It is also noteworthy that, despite increasing evidence of gene by parenting interaction in relation to substance use outcomes, little research has examined how genes and parenting interact in predicting trajectories of substance use over time. Given the mixed findings in previous studies, more research is needed, particularly longitudinal studies that include large, nationally representative samples to examine gene–parenting interaction effects, and potential variations in gene–parenting interaction effects, in predicting substance use over time. Next, conceptual and empirical evidence of gender difference in genetic and gene by environment interaction effects were briefly reviewed.

Gender Differences in Genetic and Gene–Environment Interaction Effects

Conceptually, although genes located on autosomes such as DRD4 and 5-HTTLPR generally do not vary in their structures and allele frequencies across gender, there might be gender differences in effects of these genes on developmental outcomes due to both biological and psychosocial/environmental reasons. Biologically, hormones have been suggested to affect gene expression and gender difference in hormones might lead to gender differences in genetic effects. For example, sex hormones have been suggested to affect the density of certain serotonin receptor sites and serotonin transporters in the brain. Specifically, estrogen has been shown to increase central serotonin neurotransmission and expression of 5-HTTLPR (Fink, Sumner, Rosie, Wilson, & McQueen, 1999), which may lead to gender differences in effects of 5-HTTLPR in

relation to substance use. Hormonal influences on gender-specific gene expression might be particularly salient for adolescents as adolescence has been characterized as a unique period of development that involves rapid changes related to pubertal hormones (Dahl & Hariri, 2005). In addition to pubertal hormones, gender differences in stress hormones could also play a role in gender-specific expression of genes, particularly for genes that relate to stress response and emotion regulation (e.g., 5-HTTLPR). In line of this reasoning, it has been shown that effect of 5-HTTLPR on aggression behaviors in response to stress is gender-specific. Specifically, in a sample of college students, men with the homozygous short (S/S) genotype showed increased aggression only under stress, whereas women and men carrying the long allele (S/L, with very few L/L) did not show differences in aggression in stress versus no-stress (Verona, Joiner, Johnson, & Bender, 2006).

Gender might also moderate genetic effects because psychosocial factors related to gender might impact expression of genes. There has been evidence for epigenetic effects that suggest influence of social environments on regulation and expression of genes (Meaney, 2010). For example, recent studies with rodents demonstrated that maternal care (i.e., licking and grooming behaviors) in rodents influenced expression of serotonin transporter gene in their offspring through processes that involved DNA methylation, which affected maternal caring behavior among rodent offspring (Meaney, 2010). Because of differences in gender roles and socialization for males and females (Lytton & Romney, 1991), it is possible that social environments including parenting behaviors might differ and have different meanings for males and females, which might

lead to gender differences in environmental influence on gene expression, that is, gender differences in gene by environment interaction effects.

Although conceptually plausible, gender difference in genetic effects has rarely been examined as most previous molecular genetic studies treated gender as a covariate or control variable. Studies to date that have considered gender differences in effects of DRD4 and 5-HTTLPR on psychosocial outcomes related to substance use have yielded mixed findings. For example, in a high-risk community sample of adolescents, 7-repeat allele of DRD4 was associated with greater amount of alcohol consumption and higher frequency of heavy drinking among males but not females (Laucht et al., 2007). Similarly, in a sample of adolescents living in Russia, 7-repeat allele of DRD4 was associated with higher delinquency, short temper, and thrill seeking for males, but not for females (Dmitrieva et al., 2011). However, among a sample of heavy drinking college students, 7-repeat allele of DRD4 was found to be associated with more problematic alcohol use, with the association statistically comparable across gender (Ray et al., 2008). It appears that DRD4 7-repeat allele might be a more salient risk factor for substance use related problems for males than for females. However, given the limited and inconsistent findings from previous research, more research is needed to replicate these findings before conclusions can be made regarding gender difference in effects of DRD4.

Gender difference in effects of 5-HTTLPR has also been found. Using data from the Add Health, 5-HTTLPR short allele was found to be associated with more alcohol use problems among females but not males (Vaske et al., 2012). In depression research, 5-HTTLPR short allele has been found to be associated with risk for depression among

girls and women, whereas 5-HTTLPR long allele has been found to confer vulnerability for stress and depression among boys and men (Brummett et al., 2008; Priess-Groben & Hyde, 2013). These findings suggest that effects of 5-HTTLPR on psychosocial outcomes including substance use might be dependent on gender.

In addition to gender difference in genetic main effects, there has also been some evidence of gender difference in gene–environment interaction effects in substance use related outcomes. For example, using sample from the Add Health, interaction between childhood neglect and 5-HTTLPR was found for females but not for males in predicting marijuana use (Vaske et al., 2012). Similarly, also using data from the Add Health, Li and Lee (2010) found that 5-HTTLPR interacted with childhood maltreatment in predicting antisocial behavior for girls, but not for boys. In a study of African American adolescents from rural communities, significant interaction between DRD4 and environment quality was found in predicting early sexual onset for males but not for females. Specifically, for male youth, carriers of 7-repeat allele of DRD4 were more likely to report early sexual onset in negative community environments and not to report early sexual onset in positive community environments (Kogan et al., 2014). As such, although evidence is still limited, these findings suggest that gene by environment interaction effects involving DRD4 and 5-HTTLPR might vary across gender. More research is needed to replicate these findings to better understand gender difference in genetic and gene–environment interaction effects in predicting developmental outcomes.

Control Variables

Race was considered as a control variable in all analyses in this study as race might serve as a confounding factor for the association between genetic variants and substance use trajectories due to population stratification. Population stratification refers to the presence of a systematic difference in allele frequencies between subpopulations in a population due to different ancestry. Individuals of different race/ethnicity are generally considered as different in ancestry, and with evidence that frequencies of genetic alleles differ across racial/ethnic groups (e.g., Ioannidis, Ntzani, & Trikalinos, 2004), there is concern that association between a certain genetic variant and a behavioral outcome might be spurious or biased by race if frequency of this genetic variant vary across race (Hutchison, Stallings, McGeary, & Bryan, 2004). Population stratification can also be an important concern in genetic studies on substance use. Prevalence of substance use has been demonstrated to vary across racial groups (Johnston et al., 2012). If the allele frequencies of DRD4 and 5-HTTLPR also vary across racial groups, then the associations between these genes and substance use (if they are found) might be confounded by race. As such, race was treated as a control variable in the current study.

Although self-report race/ethnicity is not an ideal measure of ancestry and controlling for self-report race/ethnicity may not be sufficient in accounting for population stratification, it is the recommended approach when more superior approaches such as genomic control and structured association methods are not available (Barnholtz-Sloan, McEvoy, Shriver & Rebbeck, 2008). Both genomic control and structured association methods involve using a set of non-candidate genetic markers to estimate

genomic ancestry and population stratification effects. Because Add Health only has data on a few genes that are potentially associated with substance use (i.e., candidate genes), these approaches were not available to account for population stratification effects in the current study. As such, although potentially not sufficient, I controlled for self-report race/ethnicity to account for potential population stratification effects.

The Current Study

The current study aimed at contributing to the extant literature of etiology of substance use by examining gene–gene interaction and gene–environment interaction effects in predicting trajectories of substance use and potential gender differences in genetic and gene by environment interaction effects, using a large-scale nationally representative sample. Specifically, the purposes of the current study were fourfold. First, this study aimed at identifying distinct trajectories of substance use from adolescence to young adulthood using a large-scale nationally representative sample. Trajectories of alcohol, cigarette, and marijuana use were examined separately to investigate potential differences in patterns of use across substances. Secondly, this study examined independent and interactive effects of genes (i.e., DRD4 and 5-HTTLPR) in predicting substance use trajectories over time. Thirdly, gene–environment interaction effects (i.e., DRD4 by parenting quality, 5-HTTLPR by parenting quality) were examined in predicting substance use trajectories. Fourthly, gender differences were examined to consider whether independent and interactive effects of DRD4 and 5-HTTLPR, as well as their interaction effects with parenting varied across gender in relation to substance use trajectories.

Building on the extant literature, it was hypothesized that distinct trajectories of substance use would be identified. Specifically, there would be a significant proportion of individuals who had never engaged in substance use, and among individuals who had initiated substance use, it was hypothesized that they would be classified into different trajectories such as developmentally-limited use trajectory, chronic or persistent use trajectory and late-onset use trajectory. Regarding genetic effects, it was hypothesized that 7-repeat allele of DRD4 and short allele of 5-HTTLPR would be associated with greater likelihood of being in substance-using trajectories, particularly the chronic or persistent use trajectory. It was also hypothesized that interaction between DRD4 and 5-HTTLPR would be found. But no specific hypothesis was proposed regarding nature of the interaction effect given limited and mixed findings from previous studies. Parenting was hypothesized to be significantly related, such that higher parenting quality would be associated with lower likelihood of being classified in substance-using trajectories. It was also hypothesized that DRD4 and 5-HTTLPR would interact with parenting quality in predicting substance use trajectory, but no specific hypothesis was proposed regarding nature of the interaction effects because of the alternative theoretical perspectives regarding nature of gene-environment interaction as well as mixed results from previous studies. Significant interaction effects (if found) would be probed to examine whether they were consistent with the diathesis-stress model or the differential susceptibility hypothesis. Likewise, no specific hypothesis was proposed regarding gender differences in genetic and gene by environment interaction effects, due to limited and mixed evidence from previous studies. As such, examination of gender differences regarding

genetic and gene by environment interaction effects in the current study was mostly exploratory.

CHAPTER IV

METHOD

Data and Procedures

This study used data from the National Longitudinal Study of Adolescent Health (Add Health). Add Health is a longitudinal study of a nationally representative sample of adolescents in grades 7-12 in the United States in 1994-1995. Participants in Add Health have been followed through adolescence and the transition to adulthood with four waves of in-home interviews which occurred at 1994/1995, 1996, 2001/2002, and 2008. Add Health includes longitudinal survey data on respondents' social, psychological and physical well-being with contextual data on the family, neighborhood, school, peer groups, and romantic relationships. In addition, Add Health also includes biological data such as DNA which was collected at wave IV for all respondents. This collection of longitudinal data in Add Health provides unique opportunities to study how biological and social environments interplay in influencing trajectories of health outcomes from adolescence to young adulthood.

Original recruitment of adolescents in Add Health involved a school-based design (Harris, 2011). A sample of 80 high schools stratified by region, urbanicity, school type, ethnic mix, and size was selected from a sampling frame derived from the Quality Education Database. For each high school selected, one of its feeder schools (typically a middle school) was identified and recruited, resulting in a sample of 132 schools. From

September 1994 until April 1995, in-school questionnaires were administered to all students in 132 participating schools. Students on school rosters and students not on rosters who completed in-school questionnaires were stratified in each school by grade and gender and about 17 students from each strata were chosen to yield a total of approximately 200 adolescents from each pair of schools, resulting in a core sample of 12,105 adolescents in grades 7-12. Add Health also included special oversamples of ethnic minority and disabled adolescents as well as a genetic sibling sample. The core sample plus the special samples produced a sample size of 20,745 adolescents at Wave I (1994-1995). In 1996, all adolescents in grades 7 through 11 in Wave I were followed up one year later for the wave II in-home interview (N = 14, 738). Wave III (transition to adulthood) data collection was conducted between August 2001 and April 2002 (N = 15, 197). In 2008, Wave IV in-home interview was conducted with 15,701 original Add Health participants.

Add Health collected four waves of data from participants in multiple age cohorts. Specifically, Add Health included participants aged between 12 (birth cohort 1983) and 21 (birth cohort 1974) at Wave I who became 13-22 years old at Wave II, 18-27 years old at Wave III, and 25-34 years old at Wave IV. The typical approach researchers use to examine developmental trajectories over time is to analyze data by examining how people change over multiple waves of data collection. However, analyzing developmental trajectories by wave as a unit of analysis for Add Health can be problematic due to methodological concerns that it would potentially ignore the wide range of age variation at each wave in Add Health. Potential differences between age or

cohort groups might lead to bias in estimation of developmental trajectories of substance use for the current study. An alternative approach is to treat Add Health as an accelerated longitudinal design (Singer & Willet, 2003; Little, 2013) in which multiple cohorts are followed over time. This design of Add Health provided an opportunity to examine developmental trajectories spanning from early adolescence to young adulthood by linking the cohorts together on the basis of age. In this sense, a long-term longitudinal study is approximated by conducting several short-term longitudinal studies of different age cohorts simultaneously. Analyzing Add Health data as an accelerated longitudinal design not only takes into account potential cohort effects but also allows for examination of developmental trajectories spanning a longer period of time.

For the purpose of this study, participants who provided genetic data at Add Health Wave IV were included. Because everyone who provided genetic data at Wave IV also participated at Wave I, this approach ensured that every individual in the sample participated in at least two waves of data collection. Individuals who did not have valid sampling weights were excluded, as data on sampling weights were needed in all analyses to provide nationally representative estimates. Given the small sample sizes for individuals who were aged 12 (birth cohort 1983, $n = 78$), 20 (birth cohort 1975, $n = 155$), and 21 (birth cohort 1974, $n = 35$) at Wave I, these individuals were also excluded. Participants who self-identified as American Indian or other-race were also excluded due to small sample sizes in these groups. This approach resulted in a final sample of 13,749 individuals for the current study. Table 1 presents the number of participants by wave, cohort, and age. As shown in Table 1, treating Add Health as an accelerated longitudinal

design provided data from age 13 to 32 for the current study to examine trajectories of substance use. Due to the nature of accelerated longitudinal design, there are missing data for each age assessment, which can be considered as planned missingness (Little, 2013) that can be handled via full information maximum likelihood estimation.

Almost all interviews were conducted at participants' home by trained research assistants. Participants completed surveys using laptop computers. Interviews were conducted using audio-CASI technology (audio-computer assisted self-interview) on laptop computers for sensitive health status and health-risk behavior questions. At Wave IV, immediately following the 90-minute interview, saliva samples were collected from all participants for buccal cell DNA. Saliva samples were then mailed by the interviewers to the Institute for Behavioral Genetics in Boulder, CO, the DNA subcontractor, where the DNA was extracted, quantified, genotyped, and stored. Complete descriptions of all data collection procedures can be found in the documentation on design features of Add Health (Harris, 2011).

Measures

Substance use. At each wave, participants responded to survey questions regarding their substance use behaviors. *Alcohol use* was measured by four items: “During the past 12 months, on how many days did you drink alcohol”, “Think of all the times you have had a drink during the past 12 months. How many drinks did you usually have each time”, “Over the past 12 months, on how many days did you drink five or more drinks in a row”, and “Over the past 12 months, on how many days did you get drunk”. Participants reported the number of days they drank or get drunk on a 7-point

scale. Response categories were 1= every day/almost every day, 2= 3-5 days per week, 3 = 1 or 2 days per week, 4= 2 or 3 days per month, 5 = once a month or less (3-12 times in the past 12 months), 6 = 1 or 2 days in the past 12 month, and 7 = never. Scores were reverse coded for these items so that higher values indicated more frequent alcohol use. Participants indicated the actual number of drinks they usually had each time, and the numbers were recoded into a 7-point scale to be in the same metric with the other three items, following the approach used by Chen & Jacobson (2012). The recoded categories were 0= none, 1= one or two drinks, 2 = 3 or 4 drinks, 3 = 5 drinks, 4 = 6 to 7 drinks, 5 = 8-10 drinks, 6 = 11-18 drinks, with more than 19 drinks coded as missing. Scores on these four items were averaged to create a composite variable capturing frequency and quantity of alcohol use, with higher scores indicating higher levels of alcohol use. Cronbach's alpha for this scale was .91 at Wave I, .92 at Wave II, .90 at Wave III, and .89 at Wave IV. *Cigarette use* was measured by two questions: "During the past 30 days, on how many days did you smoke" and "During the past 30 days, on the days you smoked, how many cigarettes did you smoke each day". Participants responded to these two questions by indicating the actual number of days they smoked (ranged from 0 to 30) and the actual number of cigarettes smoked (ranged from 0 to 40, with numbers over 40 recoded as missing). Scores on these two items were standardized and averaged to create a composite variable representing frequency and quantity of cigarette use, with higher scores indicating higher levels of cigarette use. Correlation between these two items were .77 at Wave I and Wave II and were .80 at Wave III and Wave IV. *Marijuana use* was assessed by one question: "During the past 30 days, how many times did you use

marijuana”. Respondents indicated the actual number of times they used marijuana at Wave I, II and III, and responded to a 7-point scale ranging from 0 = none to 6 = everyday/almost every day at Wave IV. The actual number of times used marijuana reported by participants at Wave I, II, and III were recoded to a 7-point scale to be consistent with the metric used at Wave IV, following the approach used by Chen & Jacobson (2012). The recoded categories were 0 = 0 times, 1 = 1 to 5 times, 2 = 6 to 10 times, 3 = 11 to 15 times, 4 = 16 to 20 times, 5 = 21 to 25 times, and 6 = 26 to 30 times, with number of times greater than 30 recoded as missing. Higher scores on this item indicated more marijuana use. Although this single item was not an ideal measure of marijuana use and might lack validity, this is common for measurement of marijuana in studies using Add Health as well as studies with other data sources (Brook et al., 2014; Tucker, de la Haye, Kennedy, Green, & Pollard, 2014).

Genotypes. DNA was extracted from saliva samples and genotyped for several polymorphisms, including the DRD4 and 5-HTTLPR polymorphisms. The dopamine D4 receptor gene (DRD4), which maps to 11p15.5, contains a 48 bp Variable Number Tandem Repeat (VNTR) polymorphism in the third exon (van Tol et al., 1992), which results in ten allelic products comprised of from 2-11 repeat units. This VNTR, which codes for the proline-rich third cytoplasmic loop of the receptor protein has been shown to affect the function of the D4 receptor in vivo: the longer variants (≥ 7 -repeat) appear to blunt the intracellular response to dopamine in vitro as compared with the shorter variants (Asghari et al., 1995). The assay (Anchordoquy et al, 2003) was a modification of an extant method (Lerman, et al., 1998). The primer sequences were forward: VIC-GCT

CAT GCT GCT GCT CTA CTG GGC; and reverse: CTG CGG GTC TGC GGT GGA GTC TGG; and yield products of 279 (2R), 327 (3R), 375 (4R), 423 (5R), 471 (6R), 519 (7R), 567 (8R), 615 (9R), 663 (10R) and 711 (11R) bp. Because other studies have grouped participants on the basis of whether they carried a long or short allele (e.g., Lerman et al., 1998; Hutchison et al., 2002) and on the basis of molecular work suggesting that the 7-repeat allele confers a functional difference in D4 receptors (Asghari et al., 1995), participants were grouped as those who carried DRD4 7-repeat allele (i.e., homozygous or heterozygous for at least one allele of ≥ 7 repeats) versus those who did not carry DRD4 7-repeat allele (i.e., both alleles < 7 repeats).

The serotonin Transporter (*SLC6A4*), which maps to 17q11.1-17q12 (Ramamoorthy et al., 1993), contains a 43 bp insertion / deletion (in/del, 5-HTTLPR) polymorphism in the 5' regulatory region of the gene (Heils et al., 1996). The in/del in the promoter appears to be associated with variations in transcriptional activity: the long variant (L) has approximately three times the expression of the short promoter (S) with the deletion (Lesch et al., 1996), although this is not a universal finding (Willeit et al., 2001, Kaiser et al., 2002). The assay for 5-HTTLPR was a modification (Anchordoquy et al., 2003) of the method of Lesch et al., (1996) using the primer sequences from Gelernter et al. (1999) forward: NED-ATG CCA GCA CCT AAC CCC TAA TGT; and reverse: GGA CCG CAA GGT GGG CGG GA, which yield products of 376 (S) or 419 (L) for the two most common alleles. The most common S and L alleles contain 14 or 16 repeat units, respectively. Extra-long alleles containing 17 (440bp), 18 (461bp), 19 (483), 20 (505) and 22 (549) repeat units were also found in the Add Health database

(Smolen et al., 2013). No functional differences have been shown between extra-long alleles and long alleles, as such extra-long alleles are grouped with long alleles (L) for analysis. Because individuals with the L/L genotype were found to have significantly higher maximal uptake of serotonin into platelets compared to those with L/S or S/S genotypes (Nobile et al., 1999, Greenberg et al., 1999), the current study, consistent with other studies (e.g., Brody et al., 2009), grouped participants based on whether they carried a short allele or not, that is, to dichotomize data to any S (S/S and S/L) vs no S (L/L) alleles.

Parenting quality. The Add Health contains a range of items related to quality of parenting. The current study used three scales that have been developed by prior Add Health researchers to measure parenting quality: maternal involvement, maternal attachment, and maternal warmth (e.g., Beaver & Belsky, 2012; Mogro-Wilson, 2008). Add Health has adolescent reports on parenting behaviors related to attachment and warmth of their residential mother and father (biological, step, adoptive, foster, etc.), as well as adolescent reports on parental involvement for both residential mother/father and non-residential biological mother/father. However, over 30% of the participants were missing data on parenting measures of residential father. Data for biological parents was only available for the involvement scale and was not available for the attachment and warmth scales. Moreover, adolescents varied in terms of the extent to which they interacted/communicated with their biological parents, and how long they had stayed with their biological parents; as such, validity of parenting measures regarding biological parents might vary among participants. Given this large amount of missing data on

paternal parenting behaviors and the limitations related to parenting measures for biological parents, I chose to only include measures of residential mothers' parenting behaviors in this study as there were much less missing data (5.6%) and multiple dimensions of parenting behaviors were measured for residential mothers. This has also been the typical approach used by other Add Health researchers (e.g., Beaver & Belsky, 2012; Mogro-Wilson, 2008).

The maternal involvement scale measured the extent to which mothers were involved in their children's life. At Wave I, adolescents indicated whether or not they and their residential mother had participated in ten activities (e.g., played a sport, gone to a movie, talked about a personal problem the adolescent was having, talked about school work, etc.) during the past month. Responses to these items (1= yes, and 0 = no) regarding activities participated with mother were summed to create a composite scale representing maternal involvement. Maternal attachment was measured by two questions. At wave I, adolescents reported on how close they felt to their residential mother and how much they thought their mother cared about them. Response options to these two questions ranged from 1= not at all to 5 = very much. Responses to these two questions were averaged to create a summary variable representing maternal attachment. During Wave I interview, adolescents also responded to three questions regarding how warm and loving their residential mother was and the overall quality of their relationship with their mother (e.g., most of the time, your mother is warm and loving toward you). Response options to these three items ranged from 1= strongly agree to 5= strongly disagree. Responses to these items were reversed coded and averaged to create a

summary variable representing maternal warmth, with higher values indicating higher warmth.

Following the approach used by Beaver and Belsky (2012), a principal components factor analysis with varimax rotation was conducted on these three parenting summary variables (i.e., maternal involvement, maternal attachment, and maternal warmth) to examine whether the variance-covariance matrix of these variables can be accounted by one single factor. Results indicated that these three variables indeed loaded on one single factor with factor loadings being .87, .87, and .49 for maternal attachment, maternal warmth, and maternal involvement, respectively, accounting for 58.17% of total variance. As such, a weighted factor score was created with standardized scores of maternal attachment, maternal warmth, and maternal involvement to create a composite measure representing parenting quality.

It is possible that some adolescents may experience, for example, high attachment and warmth but low involvement whereas others might experience high attachment and warmth as well as high involvement, and that variations in types of parenting quality may have different implications for substance use as well as gene-environment interaction effects. To explore this possibility, I conducted a latent profile analysis with maternal attachment, maternal warmth, and maternal involvement as indicators to examine whether adolescents can be classified into different groups characterized by different types of parenting quality. Results indicated that adolescents classified into different groups differed in mean levels of attachment, warmth, and involvement in the same pattern. That is, individuals who were high on warmth and attachment were also high on

involvement, whereas individuals who were low on warmth and attachment were also low on involvement. These findings indicated that parenting behaviors as measured by maternal involvement, maternal warmth, and maternal attachment did not represent different typologies of parenting quality. Instead, it can be conclude that considering parenting quality as a continuous variable measured by composite of these three subscales was a reasonable approach for the current study.

Gender and race. Participants reported their gender by responding to one question “what sex are you”. Respondents were also asked two questions regarding their race/ethnicity: “are you of Hispanic/Spanish origin”, and “what is your race”. Response options for the latter question included White, Black, Asian, American Indian, and other. Those who reported to be Hispanic were also asked to indicate their backgrounds: Mexican/Mexican American, Chicano/Chicana, Cuban, Puerto Rican, Central/South American, and other Hispanic. Similarly, those who indicated that they were Asian were also asked to report their backgrounds: Chinese, Filipino, Japanese, Asian Indian, Korean, Vietnamese, and other. Because of small sample sizes for each sub-categories of race/ethnicity (e.g., Cuban, Chinese), participants were grouped in larger racial/ethnic categories: White, Hispanic, Black, Asian, American Indian, and other race, although I recognize that there might be variations within each racial/ethnic group given individual differences in backgrounds. Participants who were American Indian or other-race were excluded due to small sample sizes, resulting in four racial/ethnic categories in the final sample: White, Hispanic, Black, and Asian. Race was dummy coded with non-Hispanic White being the reference group for subsequent analyses.

Analysis

All analyses were conducted using Mplus 7.0. Mplus has many advantaged features that are suitable for analyses in the current study. First, Mplus has the capability to take into account complex survey data such as sampling weights and clustering. Because individuals in the Add Health were clustered in schools and sampling weights were applied to ensure that the sample is nationally representative, analyses with the Add Health data need to take into account both the clustering and sampling weights in order to yield non-biased and generalizable results. As such, sampling weights and clustering by school were taken into account in all analyses in the current study. Another advantage of Mplus is the ability to run analyses using full information maximum likelihood (FIML) which is the recommended approach to analyses with data sets that include missing values (Acock, 2005). For analyses in the current study, missing data were handled via FIML which takes all available data into account. Mplus is also advanced in that it has the capability to run complex longitudinal analysis such as growth mixture modeling, which is one of the key analyses in the current study.

Identifying Trajectories of Substance Use

Growth mixture modeling (GMM) analyses were conducted to identify trajectories of alcohol, cigarette, and marijuana use from age 13 to 32. GMM is a useful tool to capture heterogeneity among individuals in their trajectories or growth curves of a certain developmental outcome over time (Muthén, & Muthén, 2000). Compared to traditional growth modeling that estimates a mean growth curve under the assumption that all individuals in the sample come from a single population, GMM takes unobserved

heterogeneity in the population into account by using latent classes, with a mean growth curve for each latent class being estimated. GMM assumes that individuals within the same latent class follow a similar growth curve over time that is distinct from individuals in other latent classes while also capturing individual variation around the mean growth curves by the estimation of growth factor variances for each class. In GMM, latent classes are indicated by different latent growth curves, that is, different latent classes differ in terms of latent growth factors (i.e., intercept, slopes).

In the current study, growth mixture models specifying different number of latent classes (from 2 to 6) were examined. Quadratic growth curves were specified for each class. Although in theory higher order growth curves were possible, models specifying higher order growth curves such as cubic growth curve did not converge. For each quadratic growth model, time score for age 13 was specified as zero, as such, the intercept represented estimated substance use at age 13. Time scores for other ages were obtained as the difference in age between measurement occasions divided by ten. For example, time score for age 14 was specified as .1, and time score for age 32 was specified as 1.9. The division is a recommended approach used to avoid large time scores which can lead to convergence problems (Muthén & Muthén, 1998-2012).

An optimal growth mixture model was selected following the approach reviewed by Wang and Bodner (2007). Model fit indices were compared across models specifying different number of latent classes. Specifically, entropy and sample size adjusted Bayesian Information Criterion (adjusted BIC) were evaluated and compared across models. Entropy is an overall indicator of classification certainty ranging from 0 to 1,

and higher values indicate better classification. Smaller value of BIC indicates better model fit. Other fit indices such as the Lo-Mendell-Rubin Likelihood Ratio Test (LMR LRT) and Akaike's Informational Criterion (AIC) have also been commonly used to evaluate growth mixture models. A significant test result of LMR LRT indicates that the K-1 model should be rejected in favor of a model with at least K classes (Lo, Mendell, & Rubin, 2001; Muthén, 2004), and smaller value of AIC indicates better model fit. However, LMR LRT tends to be significant with large sample size and simulation studies suggested that BIC outperformed these other fit indices in deciding number of classes (Nylund, Asparouhov, & Muthen, 2007). As such, the current study focused on evaluating entropy and BIC, rather than these other fit indices in deciding number of classes for growth mixture models. In addition, latent class separation (i.e., how are each class distinguishable from each other), and model interpretability (e.g., class size and meaningfulness of each class) were taken into account to determine the optimal solution regarding number of classes.

GMM analyses were conducted separately for alcohol use, cigarette use, and marijuana use given that trajectories of use of different substances and etiological processes of these substance uses might vary. To account for potential gender differences in trajectories of substance use, GMM were first conducted separately for males and females. If there were gender differences in number of classes or characteristics of growth curves identified in GMM models, subsequent analyses were analyzed separately for males and females. Otherwise, analyses were conducted with the whole sample (i.e.,

males and females combined), with gender treated as a covariate and moderator for genetic and gene–environment interaction effects.

Predicting Trajectories of Substance Use

After identifying trajectories of alcohol use, cigarette use, and marijuana use using GMM, multinomial logistic regression analyses (separately for each substance use outcomes) were conducted to examine how genes, parenting quality, and their interactions were related to likelihood of following each trajectory. Multinomial logistic regressions were conducted using the R3STEP command in Mplus, which is an automatic approach linking covariates to class membership (Muthén & Muthén, 1998-2012). Race was controlled in all analyses to take into account potential population stratification effects. Interaction effects between genes (i.e., DRD4, 5-HTTLPR) and parenting quality were evaluated by creating product terms between DRD4, 5-HTTLPR and parenting quality. Parenting quality was mean-centered before creating the product terms as recommended to avoid potential issues related to multicollinearity (Cohen, Cohen, West, & Aiken, 2003). Gender differences in effects of genes and gene–parenting interactions were also examined by creating product terms between gender and genes (e.g., gender x DRD4), and parenting quality (e.g., gender x DRD4 x parenting quality).

CHAPTER V

RESULTS

Preliminary Analysis

Add Health participants who were included in the sample for this study reported higher levels of maternal education ($t = -5.17$, $df = 18411$, $p < .001$), higher alcohol use at wave III and Wave IV ($t = -4.23$, $df = 15161$, $p < .001$, and $t = -3.48$, $df = 15686$, $p < .01$, respectively), higher cigarette use at wave III and Wave IV ($t = -2.80$, $df = 15137$, $p < .01$, and $t = -2.05$, $df = 15699$, $p < .05$, respectively), and lower marijuana use at wave I ($t = 2.35$, $df = 20135$, $p < .05$), than those who were excluded from this study. Those included in the final sample were also more likely to be White ($\chi^2 = 172.43$, $df = 3$, $p < .001$) and female ($\chi^2 = 128.30$, $df = 1$, $p < .001$). Those included in the final sample did not differ from those excluded in terms of parenting quality ($t = -1.54$, $df = 19439$, $p = .12$). As such, in addition to race, maternal education and gender were included as covariates in all analyses.

Frequencies of genetic alleles in the whole sample and by race and gender are presented in Table 2. Chi-square tests indicated that frequencies of DRD4 alleles significantly varied across race ($\chi^2 = 389.78$, $df = 3$, $p < .001$) and gender ($\chi^2 = 7.31$, $df = 1$, $p < .01$). The 7-repeat allele was more prevalent among Hispanics and Blacks than among Whites and Asians. The 7-repeat allele was also slightly more prevalent among

females than among males. Frequencies of the 5-HTTLPR alleles significantly varied across race ($\chi^2 = 874.55$, $df = 3$, $p < .001$) but did not vary across gender ($\chi^2 = 1.88$, $df = 1$, $p > .05$). The 5-HTTLPR short allele was most prevalent among Asians, followed by Hispanics, Whites, and Blacks. Chi-square tests were performed to test whether allele frequencies of DRD4 and 5-HTTLPR were in Hardy–Weinberg Equilibrium (HWE). Results indicated that distributions of DRD4 and 5-HTTLPR alleles were in HWE for the whole sample and each race/ethnicity and gender group.

Identifying Substance Use Trajectories

Trajectories of Alcohol Use. A series of growth mixture models specifying two to six classes of alcohol use trajectories were evaluated separately for males and females. Table 3 presents fit indices for these models. A review of the fit indices and the trajectories identified by each model suggested that the four-class model was optimal for both males and females, as this model had relatively smaller BIC and higher entropy, and identified alcohol use trajectories that were distinguishable from each other and interpretable from a substantive standpoint. However, the alcohol use trajectories identified from the optimal four-class models were somewhat different for males and for females.

As presented in Figure 1, four distinct trajectories of alcohol use were identified for males. An estimated 49.9% of the male sample were classified as non-drinkers/experimenters who followed a trajectory characterized by none or light alcohol use with a slight increase in use over time. 32.8% of males were classified as escalators. These individuals followed an escalating trajectory of alcohol use from early adolescence

to young adulthood in which their light alcohol use at early adolescence increased into early adulthood (although there was a trend of slight decrease in use starting age 29).

11.1% of the male sample were classified as adult-quitters. These individuals followed a trajectory characterized by moderate alcohol use in early adolescence, increase in alcohol use throughout adolescence (reaching peak use around age 22) and decline in alcohol use during young adulthood. Finally, 6.2% of males were classified as persistent heavy drinkers who followed a trajectory of persistent heavy alcohol use from early adolescence to young adulthood.

Figure 2 presents four distinct alcohol use trajectories identified for females. Similar to males, the largest group in the female sample were classified as non-drinkers/experimenters (63.0%). These females followed a trajectory characterized by none or light alcohol use with a slight increase in use over time. 21.5% of females were classified as escalators. Similar to escalators among males, these females also followed an escalating trajectory of alcohol use over time. More specifically, they started with light alcohol use at early adolescence, and increased in use over time into early adulthood (although there was a trend of slight decrease in use starting age 29). 6.2% of the female sample were classified as adult-quitters. Similar to adult-quitters among males, these females also followed a trajectory characterized by moderate alcohol use in early adolescence, increase in alcohol use throughout adolescence (reaching peak use around age 22) and decline in alcohol use during young adulthood. Different from males, a persistent heavy drinking trajectory was not identified among females. Instead, 9.3% of females were classified as developmentally-limited drinkers. These females followed a

decreasing alcohol use trajectory, characterized by moderate alcohol use during adolescence, decline in alcohol use over time, and light or none alcohol use during young adulthood.

Trajectories of Cigarette Use. A series of growth mixture models specifying two to six classes of cigarette use trajectories were evaluated separately for males and females. The four-class model was chosen as the optimal model for both males and females, as this model demonstrated relatively good model fit (see Table 4 for model fit indices for each gender group) and identified distinguishable and substantively interpretable and meaningful trajectories of cigarette use. A review of results indicated that the four-class model identified similar trajectories for males and females. For both males and females, participants were classified into four groups characterized by different trajectories: non-smokers/experimenters, escalators, adult-quitters, and persistent heavy smokers, although there appeared to be some gender differences in prevalence of being classified in each trajectory. Based on these results, growth mixture models specifying two to six classes were conducted with the whole sample (females and males combined) for cigarette use. Subsequent analyses regarding cigarette use were conducted with the whole sample with gender treated as a covariate or moderator.

Fit indices for growth mixture models of cigarette use with the whole sample are presented in Table 4. Similar to results from analyses conducted separately for males and females, a review of the fit indices and the trajectories identified by each model suggested that the four-class model was optimal for the whole sample. This model had

relatively smaller BIC and higher entropy, and identified cigarette use trajectories that were distinguishable and interpretable substantively.

As illustrated in Figure 3, four distinct trajectories of cigarette use were identified, which were similar to the trajectories identified from analyses conducted separately for males and females. This further justified combining the male and female samples for analyses regarding cigarette use. With the whole sample, 67.2% of participants were classified as non-smokers/experimenters who followed a trajectory characterized by none or light cigarette use with little change over time. 19.0% of the sample were classified as escalators. These individuals followed a trajectory of continuously increasing cigarette use from early adolescence to young adulthood. More specifically, they started cigarette smoking in early adolescence, and escalated to moderate to heavy smoking in young adulthood with no trend of decline in cigarette smoking by age 32. 6.6% of the sample were classified as adult-quitters who followed a trajectory characterized by moderate cigarette use during adolescence and continuous decline in cigarette use during young adulthood. These individuals appeared to quit cigarette use by age 31. Finally, 7.2% of the sample were classified as persistent heavy smokers who followed a trajectory of persistent heavy cigarette use from early adolescence to young adulthood.

Trajectories of Marijuana Use. A series of growth mixture models specifying two to six classes of marijuana use trajectories were evaluated separately for males and females. As was the case for alcohol use and cigarette use, the four-class model was chosen as the optimal model for both males and females, as this model demonstrated relatively small BIC and high entropy (see Table 5), and identified distinguishable and

substantively interpretable and meaningful trajectories of marijuana use. A review of results indicated that the four-class model identified similar marijuana use trajectories for males and females. For both males and females, participants were classified into four groups characterized by different trajectories: non-users/experimenters, early-escalators, late-escalators, and quitters, although there appeared to be some gender differences in prevalence of being classified in each trajectory. Based on these results, growth mixture models specifying two to six classes were conducted with the whole sample (females and males combined) for marijuana use. Subsequent analyses regarding marijuana use were conducted with the whole sample with gender treated as a covariate or moderator.

Fit indices for growth mixture models of marijuana use with the whole sample are presented in Table 5. Similar to results from analyses conducted separately for males and females, a review of the fit indices and the trajectories identified by each model suggested that the four-class model was optimal for the whole sample. This model had relatively smaller BIC and higher entropy, and identified marijuana use trajectories that were distinguishable and interpretable substantively.

As illustrated in Figure 4, four distinct trajectories of marijuana use were identified, which were similar to the trajectories identified from analyses conducted separately for males and females. This further justified combining the male and female samples for analysis regarding marijuana use. With the whole sample, 83.4% of participants were classified as non-users/experimenters who followed a trajectory characterized by persistent none or light over time. 9.2% of the sample were classified as early-escalators. These individuals followed a trajectory of continuously increasing

marijuana use from early adolescence to young adulthood. More specifically, they started with light marijuana use in early adolescence, and continuously escalated from light use in early adolescence to moderate to heavy use in young adulthood with no trend of decline in use by age 32. 4.7% of the sample were classified as late-escalators. These individuals followed a trajectory characterized by stable light marijuana use across adolescent years and slow increase in marijuana use from light use to moderate use during young adulthood. Finally, 2.6% of the sample were classified as quitters who followed a trajectory of continuously decreasing marijuana use from early adolescence to young adulthood. These individuals engaged in heavy marijuana use during early adolescence, but continuously decreased in use until they quitted marijuana at around age 31.

Predicting Substance Use Trajectories

Multinomial logistic regression analyses were conducted to examine how genes (i.e., DRD4, 5-HTTLPR), parenting quality and their interactions predicted likelihood of following substance-using trajectories. The non-users/experimenters group was treated as the reference trajectory in all multinomial logistic regressions, as this group represented the largest trajectory for all substance use outcomes. As such, regression coefficients represented likelihoods of following each substance-using trajectory relative to being in the non-users/experimenters group. Analyses were conducted separately for alcohol, cigarette, and marijuana use. For alcohol use, analyses were conducted separately for males and females as different trajectories were identified across gender. For cigarette and marijuana use, multinomial logistic regressions were conducted with the

whole sample and gender differences in genetic and gene by environment interaction effects were also examined via product terms between gender, genes, and parenting quality.

Multinomial logistic regression was first conducted to examine main effects of genes (i.e., DRD4 and 5-HTTLPR) and parenting quality. A series of regression models was then conducted to examine all possible interaction effects between genes, parenting quality, and gender, following a backward step by step approach. More specifically, starting with a model including all possible interaction terms, significant tests were examined to evaluate whether the highest order interaction term (e.g., male X DRD4 X 5-HTTLPR X parenting quality in predicting cigarette and marijuana use) should be included in the model or not. If the highest order interaction term was not significant, this interaction term would be excluded from the multinomial regression model, and a new regression model would be conducted to evaluate the next highest order interaction term. If a higher order interaction term was significant, any lower order interaction terms and main effects (even if they were non-significant) that composited the higher order interaction term were kept in the model following the principle of marginality (Nelder, 1977). A series of multinomial logistic regression models were conducted following this step by step approach to establish a final model for evaluation of interaction effects.

Predicting Alcohol Use Trajectories. Multinomial logistic regressions were conducted separately for males and females in predicting alcohol use trajectories. Table 6 presents coefficients from models predicting alcohol use trajectories among males. In terms of main effects (see Model I in table 6), results indicated that DRD4 genotype was

not associated with likelihood of following any alcohol-using trajectories relative to the experimenters trajectory. However, consistent with prediction, 5-HTTLPR short allele was associated with higher likelihood of following the escalators' trajectory and the persistent heavy drinkers' trajectory, relative to the experimenters trajectory. Higher parenting quality was associated with greater likelihood of following the escalators trajectory and lower likelihood of following the adult-quitters trajectory and the persistent heavy drinkers trajectory, relative to the experimenters trajectory. Regarding interaction effects (see Model II in table 6), results indicated that there were significant interactions between 5-HTTLPR and parenting quality in relation to likelihood of following the persistent heavy drinkers trajectory. As illustrated in Figure 5, higher parenting quality was significantly associated with lower likelihood of following the persistent heavy drinkers trajectory among males carrying the 5-HTTLPR short allele ($B = -.31, p < .001$), but was not associated with likelihood of following the persistent heavy drinkers trajectory among males who did not carry the 5-HTTLPR short allele ($B = -.01, p > .10$). A closer review of the interaction effect as illustrated in Figure 5 indicated that males who carried the 5-HTTLPR short allele were at higher risk of following the persistent heavy drinkers trajectory when parenting quality was low, but were at lower risk of following the persistent heavy drinkers trajectory when parenting quality was high, compared to males who did not carry the 5-HTTLPR short allele. Consistent with the differential susceptibility perspective, this interaction effect suggested that those who carried the 5-HTTLPR short allele were more susceptible to the influence of both high and low parenting quality.

Coefficients from multinomial logistic regression models predicting alcohol use trajectories among females are presented in Table 7. Similar to findings for males, the DRD4 genotype was not associated with likelihood of following any alcohol-using trajectories among females. Opposite to prediction, the 5-HTTLPR short allele was associated with lower likelihood of following the developmentally-limited drinkers trajectory among females, relative to the experimenters trajectory. Higher parenting quality was associated with lower likelihood of following the developmentally-limited drinkers trajectory and the adult-quitters trajectory, but was not associated with the likelihood of following the escalators trajectory. Results also revealed significant three-way interaction effects between DRD4, 5-HTTLPR, and parenting quality in predicting likelihood of following the escalators trajectory among females. As illustrated in Figure 6, the nature of parenting quality by 5-HTTLPR interaction effects differed between females who carried the 7-repeat allele of DRD4 and those who did not carry the 7-repeat allele of DRD4. As shown in Figure 6 Panel A, among females who did not carry 7-repeat allele of DRD4, parenting quality was associated with lower risk of following the escalators trajectory for non-carriers of 5-HTTLPR short allele ($B = -.09, p > .10$), but was associated with higher risk of following the escalators trajectory for carriers of 5-HTTLPR short allele ($B = .05, p > .10$), although both associations were not statistically significant. On the contrary, among females who carried 7-repeat allele of DRD4 (see Figure 6 Panel B), parenting quality was associated with higher risk of following the escalators trajectory for non-carriers of 5-HTTLPR short allele ($B = .14, p > .10$), but was associated with lower risk of following the escalators trajectory for carriers of 5-

HTTLPR short allele ($B = -.07, p > .10$), although both associations were also not statistically significant. These results suggested that for females the nature of 5-HTTLPR by parenting quality interaction effect was dependent on DRD4 genotype.

Predicting Cigarette Use Trajectories. Coefficients from multinomial logistic regression models predicting cigarette use trajectories with the whole sample are presented in Table 8. In terms of main effects, DRD4 was not significantly associated with likelihood of following any cigarette-using trajectories. Contrary to prediction, 5-HTTLPR short allele was associated with lower risk of following the escalators trajectory of cigarette use. Consistent with prediction, parenting quality was associated with lower risk of following any of the cigarette-using trajectories (i.e., escalators, adult-quitters, persistent heavy smokers) relative to the non-smokers/experimenters trajectory.

Results revealed significant interaction effects between DRD4 and 5-HTTLPR in predicting likelihood of following the escalators trajectory of cigarette use (see Model II in Table 8). As illustrated in Figure 7, among those who did not carry 7-repeat allele of DRD4, 5-HTTLPR short allele was associated with lower risk of following the escalators trajectory ($B = -.30, p < .05$), whereas among those who carried the 7-repeat allele of DRD4, 5-HTTLPR was not associated with likelihood of following the escalators trajectory ($B = -.07, p > .10$), relative to the non-smokers/experimenters trajectory. To view this interaction effect in another way, DRD4 7-repeat allele was not associated with likelihood of following the escalators trajectory among individuals who did not carry 5-HTTLPR short allele ($B = -.07, p > .10$), whereas DRD4 7-repeat allele was associated with higher risk of following the escalators trajectory among carriers of 5-HTTLPR short

allele ($B = .20, p < .05$). These results suggested that genetic effects of DRD4 and 5-HTTLPR in relation to cigarette use trajectories need to be understood with the two genes considered in combination.

Results also revealed significant a three-way interaction between 5-HTTLPR, parenting quality, and gender in predicting likelihood of following the escalators trajectory of cigarette use. As illustrated in Figure 8, the nature of interaction effects between parenting quality and 5-HTTLPR differed across gender. For females (see Figure 8 Panel A), higher parenting quality was significantly associated with lower likelihood of following the escalators trajectory among those who did not carry the 5-HTTLPR short allele ($B = -.13, p < .01$), whereas parenting quality was not associated with risk of following the escalators trajectory among carriers of the 5-HTTLPR short allele ($B = -.02, p > .10$). On the contrary, for males (see Figure 8 Panel B), higher parenting quality was not associated with likelihood of following the escalators trajectory among those who did not carry the 5-HTTLPR short allele ($B = -.05, p > .10$), whereas parenting quality was significantly associated with lower risk of following the escalators trajectory among carriers of the 5-HTTLPR short allele ($B = -.08, p < .05$).

Predicting Marijuana Use Trajectories. Coefficients from multinomial logistic regression models predicting marijuana use trajectories with the whole sample are presented in Table 9. In terms of main effects (see Model I in Table 9), contrary to prediction, neither DRD4 nor 5-HTTLPR was significantly associated with risk of following marijuana-using trajectories. Consistent with prediction, higher parenting quality was significantly associated with lower likelihood of following any

marijuana-using trajectories (i.e., early escalators, late escalators, quitters) relative to the non-users/experimenters trajectory. In terms of interaction effects, results indicated that gender moderated the effects of DRD4 and 5-HTTLPR in predicting likelihood of following the early-escalators trajectory of marijuana use (see Model II in Table 9). As illustrated in Figure 9, DRD4 7-repeat allele was not significantly associated with likelihood of following the early-escalators trajectory among females ($B = .22, p > .10$), whereas DRD4 7-repeat allele was significantly associated with lower risk of following the early-escalators trajectory among males ($B = -.26, p < .05$). As shown in Figure 10, 5-HTTLPR short allele was associated with lower likelihood of following the early-escalators trajectory of marijuana use among females ($B = -.34, p < .10$), whereas 5-HTTLPR genotype was not associated with risk of following the early-escalators trajectory among males ($B = .03, p > .10$). These results suggested that effects of DRD4 and 5-HTTLPR on marijuana use trajectories were dependent on gender.

CHAPTER VI

DISCUSSION

The primary goal of this study was to identify distinct trajectories of substance use from early adolescence to young adulthood and to examine genetic and gene–environment interaction effects in predicting substance use trajectories. Moreover, this study explored potential gender differences in trajectories of substance use and genetic and gene–environment interaction effects. Distinct trajectories were identified for alcohol, cigarette, and marijuana use, with alcohol use trajectories differing for males and females, and trajectories of cigarette and marijuana use being similar across gender. The results indicated significant gene–gene interaction and gene–environment interaction effects, as well as gender differences in these effects, in predicting individual’s likelihood of following different substance use trajectories.

Trajectories of Substance Use

Four distinct trajectories of alcohol use were identified for both male and female participants, however the nature of those trajectories varied by gender. Consistent with hypotheses and previous findings, for both male and female participants the largest group was classified as non-drinkers/experimenters characterized by none or light alcohol use over time. Also consistent with hypotheses and the literature, a trajectory of persistent heavy alcohol use (for males) and a trajectory of developmentally–limited alcohol use (for females) were identified. For both males and females, this study identified

an escalating alcohol use trajectory characterized by none or light alcohol use during early adolescence and increase in use over time. This trajectory is conceptually consistent with the late-onset trajectory found in previous studies (Chassin et al., 2002). An adult-quitting trajectory of alcohol use characterized by moderate alcohol use during adolescence and decrease in use throughout young adulthood was also identified for males and females in this study, which has not been reported in the literature. This is likely because most previous studies did not include data for alcohol use with such a wide age range as this study. The adult-quitters trajectory captured a decline in alcohol use starting around age 25, which could not have been captured in previous studies that examined trajectories of alcohol use up to younger than age 25 (e.g., Jackson et al., 2008).

Consistent with hypotheses, this study identified four distinct trajectories of cigarette use that were similar for males and females. Participants were classified as non-smokers/experimenters characterized by none or light cigarette use over time, escalators characterized by increasing cigarette use from early adolescence to young adulthood, persistent heavy smokers who engaged in persistent heavy cigarette use over time, and adult-quitters who engaged in moderate cigarette use throughout adolescence but started quitting cigarette smoking in adulthood. These trajectories are largely consistent with those found in the literature. For example, using a community sample, Tucker et al. (2005) examined trajectories of smoking from age 13 to 23 and classified individuals as triers, steady increasers, and stable highs, which were similar to the non-smokers/experimenters, escalators, and persistent heavy smokers identified in this study. The adult-quitting trajectory of cigarette use identified in this study is similar to the

quitters trajectory identified by Costello and colleagues (Costello, Dierker, Jones, & Rose, 2008).

Four distinct trajectories that were similar for males and females were also identified for marijuana use. Consistent with prediction and previous findings, majority of the participants were classified as non-users/experimenters of marijuana use. A group of participants were classified as early escalators characterized by increase in marijuana use from early adolescence to young adulthood, which is consistent with the steady increasers group identified by Tucker et al. (2005). A late-escalators trajectory characterized by light marijuana use throughout adolescence and increase in marijuana use during adulthood was also identified. This trajectory has not been reported in the literature, which is not surprising as most previous studies examining trajectories of marijuana use focused on shorter developmental period of time than this study (Flory et al., 2004; Tucker et al., 2005; Windle & Wiesner, 2004). Studies that only examined trajectories of marijuana use during adolescence or from adolescence to early 20s might have labeled this group of individuals as stable light marijuana users (e.g., Tucker et al., 2005), failing to capture the increase in marijuana use among this group of individuals during adulthood. A small group of participants were classified as quitters who engaged in relatively heavy marijuana use during adolescence with decrease in use throughout adulthood. A similar decreasers/quitters group was also identified in a study that examined trajectory of marijuana use from adolescence to age 37 (Brook, Zhang, & Brook, 2011). However, in other studies that examined trajectories of marijuana use

within a smaller age range, this group might have been classified as early high users (Tucker et al., 2005) or chronic high users (Windle & Wiesner, 2004).

These results provided information on similarities and differences in trajectories of use across alcohol, cigarette, and marijuana. Consistent with hypothesis and previous findings, for each type of substance use examined, the normative trajectory (representing the largest group of individuals) involved persistent none or light substance use throughout adolescence and young adulthood. An escalating trajectory was identified for each type of substance use, representing the second largest group for alcohol, cigarette, and marijuana use. Although these escalators only engaged in light substance use during early adolescence, they increased their use and reached moderate to heavy substance use during adulthood. As such, this escalating trajectory might represent a relatively high-risk trajectory that associates with adverse developmental outcomes in adulthood. Alternatively, this escalating trajectory may be considered as relatively benign or less maladaptive, as individuals who follow this trajectory engaged in substance use when they were becoming of age, transitioning to college, and joining the workforce, which may be relatively normative during this developmental period of time. As such, whether this trajectory confers risk for individual's well-being needs to be further understood by studies examining association between this trajectory and outcomes in adulthood.

Results revealed some differences in trajectories of use for different types of substance. Specifically, a persistent heavy use trajectory was identified for both alcohol use (for males) and cigarette use, but was not found for marijuana use. Moreover, a late-escalating trajectory was found for marijuana use but not for alcohol use and

cigarette use. A quitting trajectory that can be considered as representing a “maturing out” pattern was found for each type of substance use, however, for alcohol and cigarette use, decrease/quitting of use started in adulthood, whereas for marijuana use decrease/quitting started during adolescence. These results suggested that developmental trajectories of substance use might vary depending on the specific type of substance use, although some common trajectories can be identified across types of substance use, illustrating the importance of considering use of different substances separately.

Consistent with the literature, trajectories of substance use identified in this study were largely similar across gender, although males and females differed in their likelihood of following different trajectories of substance use. Results indicated that similar trajectories of cigarette and marijuana use could be identified for males and females. But males were more likely to be classified into the escalating trajectory of cigarette use relative to the non-smokers/experimenters trajectory than females. Males were also more likely to be classified in to all marijuana–using trajectories relative to the non-users/experimenters trajectory than females, reflecting greater use of marijuana among males from adolescence to adulthood. As for alcohol use, four distinct trajectories were identified for both males and females, with three of the trajectories (i.e., non-drinkers/experimenters, adult-quitters, escalators) being similar across gender. One gender difference related to trajectories of alcohol use found in this study was that a persistent heavy alcohol use trajectory was identified for males whereas a developmentally–limited alcohol use trajectory was identified for females. These findings are consistent with the general findings from previous studies that males on

average are at greater risk for substance use than females. Building upon previous studies, these findings suggest that overall similar trajectories of substance use can be identified for males and females from early adolescence to young adulthood, but there are gender difference in likelihood of following different trajectories, even after controlling for race, maternal education, genetic risk, and parenting quality. As such, gender is an important factor to consider in understanding substance use over time.

Genetic and Environmental Effects on Trajectories of Substance Use

Genetic and Environmental Main Effects. Overall, parenting quality was consistently associated with lower risk of following substance–using trajectories. This is consistent with hypothesis and findings from previous literature that suggest a positive role of supportive parenting in reducing risk for substance use. One unexpected finding is that parenting quality was associated with greater likelihood of following the escalating trajectory of alcohol use among males. Male participants who followed the escalating trajectory engaged in light alcohol use during adolescence. Alcohol use is relatively widespread among males during adolescence and typically occurs in social settings with peers. Given evidence that social drinking is associated with greater social adjustment (Bondy, 1996), these male participants may have had better well-being compared to those who did not engage in alcohol use at all and those who engaged in higher levels of alcohol use during adolescence. To the extent that this association exists, then parenting quality, measured during adolescence, would be associated with greater likelihood of following the escalating trajectory of alcohol use and may be indicative of better adjustment during adolescence. It may also be that this escalating trajectory of alcohol

use is not maladaptive for males and thus parenting quality is positively related. Further research is needed to replicate and understand this unexpected association. Despite this one unexpected finding, results from this study build on previous literature and demonstrate that parenting quality experienced during adolescence plays an important role in predicting individual's trajectories of substance use from early adolescence throughout young adulthood.

There was no significant main effect of DRD4 genotype on trajectories of alcohol, cigarette, and marijuana use. That is, 7-repeat allele of DRD4 in and of itself did not pose increased risk for following substance-using trajectories, above and beyond gender, race, maternal education, and parenting quality. This is consistent with previous studies that also failed to find an association between DRD4 genotype and substance use (Creemers et al., 2011; Hopfer et al., 2005) but also contradicts other studies finding such an association (Bobadilla et al., 2013; Laucht et al., 2007). Previous studies linking DRD4 to substance use outcomes tended to use regional or at-risk samples of individuals and operationalized substance use outcomes as status or rates of substance use at a certain developmental time, whereas this study used a nationally representative sample and considered trajectories of substance use from early adolescence to young adulthood as outcomes. These differences in sample characteristics and operationalization of substance use outcomes between this study and previous studies might have led to the discrepancy in findings. Moreover, the null finding of main effect of DRD4 is not surprising as genetic main effects are rarely found given the notion that substance use behaviors are multiply determined by genetic and environmental factors (Dick, 2011).

As such, it is expected that DRD4 functions in combination with other genes and environmental factors in influencing trajectories of substance use.

Consistent with prediction, 5-HTTLPR short allele was associated with greater likelihood of following the escalators and persistent heavy use trajectory of alcohol use among males. This is consistent with previous findings that short allele of 5-HTTLPR was associated with higher levels of substance use among adolescents (Brody et al., 2009; Merenäkk et al., 2011) and more alcohol use problems in a sample of young adults in Add Health (Vaske et al., 2012). However, 5-HTTLPR short allele was associated with lower likelihood of following the developmentally-limited trajectory of alcohol use for females, which is consistent with previous finding that suggested protective role of 5-HTTLPR short allele against alcohol use (Olsson et al., 2005). This finding seemingly contradicted the hypothesis that short allele of 5-HTTLPR would be associated with higher risk for substance use. However, it may be that following a developmentally-limited trajectory of alcohol use is relatively normative for females and does not place females at greater risk for adverse developmental outcomes and thus may not be considered as a risk trajectory of alcohol use. Rather, given that engaging in alcohol use is common during adolescence and emerging adulthood and alcohol use during this developmental period typically occurs in social settings with peers, developmentally-limited alcohol use among females could be considered as socially normative and adjusted. On the other hand, females who have internalizing problems such as anxiety and depression may be less likely to engage in alcohol use during adolescence due to impaired social interactions and less opportunity for alcohol

experimentation in social settings. Previous research has shown that individuals, particularly females, with a short allele of 5-HTTLPR are at greater risk for anxiety and depression compared to those without a short allele of 5-HTTLPR (Lesch et al., 1996; Peterson et al., 2012). As such, that females with 5-HTTLPR short allele were less likely to follow the developmentally-limited trajectory of alcohol use may be a result of their higher risk for anxiety and depression during adolescence. Despite seeming contradiction in effects of 5-HTTLPR short allele, it remains possible that 5-HTTLPR short allele is protective for some serotonin-related outcomes, yet create risk for other serotonin-related outcomes, depending on the complex pathways underlying specific outcomes. Future research, such as studies that take into account related psychosocial factors such as anxiety and depression that may underlie substance use, is needed to better understand the influence of 5-HTTLPR on substance use outcomes.

5-HTTLPR short allele was found to be associated with lower likelihood of following the escalating trajectory of cigarette use for the whole sample. This is consistent with previous findings that the LL 5-HTTLPR genotype was more prevalent among smokers than non-smokers (Ishikawa et al., 1999; Kremer et al., 2005). Individuals with LL genotype have relatively lower levels of intrasynaptic serotonin due to higher levels of serotonin transporter expression and higher efficiency of serotonin reuptake (Lesch et al., 1996), and therefore may show lower capability of inhibition and a greater impulse toward cigarette use compared to individuals with SS or SL genotypes. Conflicting findings regarding association between 5-HTTLPR and smoking behaviors have also been reported in the literature. Some studies found that 5-HTTLPR short allele

was associated with increased risk for smoking (Gerra et al., 2005), and others reported no association between 5-HTTLPR and cigarette use (Lerman et al., 1998).

It may be that discrepancy in association between 5-HTTLPR and smoking is in part driven by different personality characteristics of smokers in different samples. For example, short allele of 5-HTTLPR was associated with more smoking among those high in neuroticism (Hu et al., 2000), whereas long allele of 5-HTTLPR was associated with more smoking among those high in novelty seeking (Kremer et al., 2005). Thus, it may be useful to consider characteristics of smokers, particularly their personality traits in understanding genetic effects on smoking, as differences in characteristics such as personality traits may have implications for different reasons, motivations, or mechanisms underlying smoking behaviors and it is possible that genetic effects vary depending on the mechanisms underlying cigarette use. It may be that individuals following the escalating trajectory of cigarette use scored relatively high on novelty or sensation seeking rather than high on neuroticism as this group of individuals included more males than females in this study. As such, LL 5-HTTLPR genotype, rather than short allele of 5-HTTLPR, conferred risk for escalating cigarette use in this study.

Gene–Gene Interaction Effects. DRD4 and 5-HTTLPR significantly interacted in predicting individual’s likelihood of following the escalating trajectory of cigarette use. The pattern of interaction suggested that LL 5-HTTLPR genotype was associated with greater risk for escalating cigarette use when 7-repeat allele of DRD4 was not present. Individuals who did not carry 7-repeat allele of DRD4 and short allele of 5-HTTLPR were at greatest risk for escalating cigarette use. This pattern of DRD4 by 5-HTTLPR

interaction effect is consistent with those found in Skowronek et al. (2006). Specifically, using a high-risk community sample of adolescents, Skowronek et al. found that females with the LL 5-HTTLPR genotype and without 7-repeat allele of DRD4 engaged in higher levels of smoking and drinking compared to females with any other genotypes.

These results are consistent with the notion that the serotonergic system and the dopaminergic system functionally interact in influencing phenotypic development (Carver, Johnson, & Joormann, 2009; Daw et al., 2002; Kapur & Remington, 1996; Wong, Feng, & Teo, 1995). It has been suggested that a balance between the serotonergic and the dopaminergic systems is optimal, and imbalance between these two systems (e.g., low serotonergic neurotransmission combined with high dopaminergic neurotransmission) may lead to risk for substance use. Individuals with LL 5-HTTLPR genotype have lower levels of serotonergic neurotransmission compared to individuals with SS or SL 5-HTTLPR genotypes due to higher efficiency of serotonin reuptake (Lesch et al., 1996). It has been argued that carriers of 7-repeat allele of DRD4 may experience stronger down-regulation of dopamine release during substance-induced dopamine activation, which can lead to more substance use among these individuals in order to reach higher levels of dopamine release and thus higher levels of reward and sensation, compared to non-carriers of 7-repeat allele of DRD4 (Skowronek et al., 2006). As such, individuals without 7-repeat allele of DRD4 may have normative or high levels of dopaminergic neurotransmission functioning as they are less vulnerable for down-regulation of dopamine release during substance use such as smoking. Individuals with LL 5-HTTLPR genotype in combination with non-7-repeat DRD4 thus have low

serotonergic transmission but high dopaminergic transmission. This imbalance in the two systems may lead to increased risk for substance use, that is, to follow the escalating trajectory of cigarette use in this case. These findings suggest that genes involved in the dopaminergic and serotonergic systems such as DRD4 and 5-HTTLPR need to be considered in combination to understand their effects in relation to substance use outcomes.

Gene–Environment Interaction Effects. Consistent with prediction, results revealed significant gene–environment interaction effects in predicting trajectories of substance use. 5-HTTLPR significantly interacted with parenting quality in predicting likelihood of following the persistent heavy alcohol use trajectory among males. The pattern of this interaction effect was consistent with the differential susceptibility hypothesis, that is, males with a short allele of 5-HTTLPR was more susceptible to influence of parenting quality in a “for better and for worse” pattern. Compared to non-carriers of 5-HTTLPR short allele, males with a short allele of 5-HTTLPR were at greater risk for following the persistent heavy alcohol use trajectory when they reported experiencing lower levels of parenting quality, but were at lower risk when experienced higher parenting quality. Previous studies have found similar patterns of interaction effects between 5-HTTLPR and parenting in relation to psychosocial outcomes such as neuroticism (Pluess et al., 2010) and positive affect (Hankin et al., 2011). However, this study is the first to provide evidence that 5-HTTLPR and parenting interact in predicting trajectory of alcohol use over time, with short allele of 5-HTTLPR being a susceptibility/plasticity genotype.

Results also revealed significant three-way interaction effects between DRD4, 5-HTTLPR, and parenting quality in predicting likelihood of following the escalators trajectory of alcohol use among females. For females, the nature of interaction between 5-HTTLPR and parenting quality was dependent on the genotype of DRD4. For females who carried the 7-repeat allele of DRD4, the nature of the interaction between 5-HTTLPR and parenting quality was consistent with the differential susceptibility hypothesis with the short allele of 5-HTTLPR as the susceptible/plastic allele, consistent with the pattern of 5-HTTLPR by parenting interaction for males as discussed above. However, for females who did not carry the 7-repeat allele of DRD4, interaction between 5-HTTLPR and parenting quality was in the opposite pattern. The nature of the interaction effect was still consistent with the differential susceptibility perspective, but the LL 5-HTTLPR genotype was the susceptible/plastic genotype. That is, for females who did not carry the 7-repeat allele of DRD4, those with LL 5-HTTLPR genotype were at greater risk of following the escalators trajectory of alcohol use when experienced low parenting quality, but was at lower risk when experienced high parenting quality, compared to those with 5-HTTLPR short allele.

These results are consistent with the idea discussed above that an imbalance between the serotonergic and the dopaminergic systems may lead to risk for substance use. In this case, females who carried 7-repeat allele of DRD4 and short allele of 5-HTTLPR may had low dopaminergic neurotransmission but high serotonergic neurotransmission, whereas females who did not carry 7-repeat allele of DRD4 with LL 5-HTTLPR genotype may had high dopaminergic neurotransmission but low

serotonergic transmission, both of which can be considered as an imbalance between the serotonergic and the dopaminergic systems. Females who were at an imbalance between the two systems were at greater risk for escalating alcohol use when experienced poor parenting quality but were at lower risk when experienced high parenting quality. These findings suggest that an imbalance between the serotonergic and the dopaminergic systems may confer greater susceptibility to the influence of both poor and high parenting quality in predicting trajectory of alcohol use.

A significant three-way interaction between 5-HTTLPR, parenting quality, and gender was also found for the escalating trajectory of cigarette use. For females, the interaction effect was consistent with the diathesis-distress/dual risk perspective, with the LL 5-HTTLPR genotype as a genetic risk factor. That is, compared to females who carried short allele of 5-HTTLPR, females with LL 5-HTTLPR genotype were at greater risk for escalating cigarette use particularly when experienced poor parenting quality. For males, those who carried the short allele of 5-HTTLPR were at lower risk for escalating cigarette use particularly when experienced high parenting quality, an interaction effect in the pattern of “dual advantages”. These findings suggested that the LL 5-HTTLPR genotype may confer greater vulnerability for cigarette use for females who experienced poor parenting quality, whereas short allele of 5-HTTLPR may confer greater susceptibility to positive influence of high parenting quality for males in relation to cigarette use. Interaction effects between 5-HTTLPR and environmental factors in relation to substance related outcomes have also been found in previous studies, and findings regarding gender differences in nature of interaction effects have been mixed

(Covault et al., 2007; Peterson et al., 2012). Nevertheless, these findings supported the notion that genetic effect of 5-HTTLPR is dependent on gender and context (Stoltenberg, Christ, & Highland, 2012).

One possible interpretation for this 5-HTTLPR by parenting quality by gender interaction effect is that females and males may differ in their mechanisms underlying cigarette use. Due to social norms around smoking, cigarette use may be associated with more aversive social cues or punishment for females, whereas whether cigarette smoking is associated with risk is more ambiguous for males. As such, cigarette use for females may be influenced by how well they process or perceive punishment or aversive cues associated with smoking, whereas cigarette use for males is more of a process of decision making under ambiguity of risk associated with smoking. It has been suggested that the LL 5-HTTLPR genotype is associated with less sensitivity to punishment and aversive cues (Blair et al., 2008), and that short allele of 5-HTTLPR is associated with better decision making under ambiguous conditions for males (Stoltenberg & Vandever, 2010). Given that parenting has been suggested to be associated with individuals' perceived risk associated with substance use (Miller, Chomeynova, & Beck, 2009), it may be that females with the LL 5-HTTLPR genotype were less sensitive to punishment and risk associated with cigarette use particularly when experienced poor parenting quality, which placed them at elevated risk for escalating cigarette use. Since parenting has also been associated with individual's decision making competence (Udell, Bannon, & McKay, 2008), it may be that for males with short allele of 5-HTTLPR, experiencing high

parenting quality amplified their capabilities of making good decisions against cigarette use, which made them less likely to follow the escalating trajectory of cigarette use.

Taken together, findings from this study provided support for gene–environment interactions in relation to trajectories of substance use and indicated that nature of gene by environment interaction effects may vary across individuals. Regardless of the nature of interaction effects, these findings are consistent with Gottlieb’s and Sameroff’s theoretical perspectives that genes and environments interact in influencing development over time. That is, both genes and environmental factors play important roles in influencing substance use over time, and that effects of environment (i.e., parenting quality) is dependent on individual’s genotype, and vice versa. Moreover, findings of gene–environment interaction effects that were consistent with the differential susceptibility perspective challenged the idea of genetically determined reaction range and provided support for the conceptualization of norm of reaction in biology. That is, genes do not provide limits for potential developmental functioning, rather, genetic effects are conditioned on environmental influences. Individuals predisposed to genetic risk might function even better than those not predisposed to genetic risk under good enough environmental conditions. Although gene–environment interaction effects found in this study that were in the patterns of dual risk or dual advantage did not provide support for the norm of reaction, they cannot be viewed as supporting the idea of reaction range either, as it might be that the measure of parenting quality in this study did not capture a range of environmental quality that was wide enough.

Gender Differences in Genetic Effects. Significant gender differences in genetic effects were apparent in this study. DRD4 genotype was associated with likelihood of following the early escalators trajectory of marijuana use for males, whereas there was no association between DRD4 and trajectory of marijuana use for females. On the contrary, 5-HTTLPR genotype was associated with likelihood of being in the early escalating trajectory of marijuana use for females, whereas there was no association between 5-HTTLPR and trajectory of marijuana use for males. These findings are consistent with previous studies suggesting that genetic effects of DRD4 may be more salient for substance use related outcomes for males than for females (Laucht et al., 2007; Dmitrieva et al., 2011), whereas 5-HTTLPR may be more influential for females than for males. However, the reason for gender differences in the association between genetic variants and substance use is not yet understood.

As noted in the introduction, gender differences in genetic effects may be due to gender differences in biological as well as psychosocial factors. Biologically, it may be that gender moderated genetic effects on marijuana use due to ways in which gender differences in hormone levels affect gene expression and serotonin and dopamine functioning (Becker, 1999; Fink et al., 1999). That females have higher dopamine release and dopamine receptor levels than males (Kaasinen, 2001; Riccardi et al., 2006) may contribute to gender difference in effects of DRD4. It may also be that the dopaminergic system associated with reward processing is more salient for males, whereas the serotonergic system associated with behavioral inhibition, mood, and affect is more relevant for females, in relation to substance use. Understanding biological

mechanisms for gender differences in genetic effects on substance use remain an important direction for future research.

These gender differences in genetic effects may also be due to ways in which males and females may experience substance use including marijuana use differently. For instance, substance use is more likely to comorbid with externalizing problems in boys, whereas it is more likely to co-occur with internalizing problems in girls (Armstrong & Costello, 2002; Su, Supple, & Stein, 2014). This gender difference in comorbid behaviors associated with substance use may implicate relevance of different genetic variants, as DRD4 has been implicated in externalizing problems such as delinquency and antisocial behaviors (Beaver et al., 2007), whereas 5-HTTLPR has been reported to be influential for internalizing problems such as anxiety and depression (Karg, Burmeister, Shedden, & Sen, 2011). Future research that takes into account comorbid internalizing and externalizing problems is needed to better understand gender differences in genetic effects on substance use.

Variations in Genetic Effects Depending on Nature of Substance Use Trajectories

It is noteworthy that genetic effects on trajectories of substance use may vary depending on nature of the trajectory. Overall, significant effects involving genes were largely found for trajectories that can be considered to be more problematic, such as persistent heavy alcohol use, escalating cigarette use, and early escalating marijuana use. These findings are consistent with findings from Vaske et al. (2013), as well as Moffit's (1993) proposition that problematic trajectories such as life-course persistent antisocial behavior is more likely to be influenced by individual predispositions such as genotypes,

whereas other trajectories that are developmentally–limited or less problematic are more likely to be influenced by environmental factors.

That genetic effects may vary depending on the specific nature of substance use trajectory have important implications for understanding genetic effects on substance use. In particular, this may shed light on understanding the inconsistent findings regarding genetic effects on substance use found in the literature. Previous studies that examined genetic effects on substance use varied in many aspects such as sample characteristics, study designs, and operationalization of substance use outcomes. It may be that participants in these studies differed in their potential trajectories of substance use over time, and this heterogeneity in trajectories of substance use among participants within the same study and across studies may have led to different findings regarding genetic effects. Findings from this study demonstrate the importance of taking into account the heterogeneity of substance use trajectories in understanding genetic effects on substance use behaviors.

Limitations and Contributions

Several limitations of this study should be noted. First, this study used a biallelic genotyping of the 5-HTTLPR based on long and short variants. Although this approach is commonly used in previous studies, it has been suggested that a SNP (rs25531, A/G) in the long variant of the 5-HTTLPR polymorphism might have functional significance such that the common LA allele is associated with higher basal activity, whereas the less common LG allele has transcriptional activity no greater than the short allele (Hu et al., 2005). This study did not examine effects of 5-HTTLPR using this triallelic genotyping

based on LA, LG, and short variants because triallelic genotyping of 5-HTTLPR was not available in the dataset used for this study. Future studies need to consider triallelic genotyping of 5-HTTLPR to replicate findings from this study. Second, although this study included a racially/ethnically diverse sample, separate analysis was not conducted to examine potential racial/ethnic difference in patterns of substance use trajectories due to variations in sample sizes across racial/ethnic groups. Moreover, this study controlled for race/ethnicity as a way to account for potential population stratification effects but failed to examine potential racial/ethnic differences in genetic and gene–environment interaction effects in predicting trajectories of substance use as it was beyond the scope of this study. Given the notion that genetic effects may vary across race (Humphreys, Scheeringa, & Drury, 2014), future research is needed to examine whether genetic effects on substance use trajectories vary across race/ethnicity. Another limitation is that this study did not control for psychosocial factors such as depression, anxiety, antisocial behaviors that may co-occur with substance use and personality traits such as sensation seeking and neuroticism that may underlie substance use. Future research that takes into account these factors may help better understand genetic and environmental influences on the heterogeneity of substance use trajectories.

Despite these limitations, this study makes significant contributions to the literature in several aspects. First, this is the first study to examine heterogeneity in trajectories of substance use with such a wide developmental period (from age 13 to age 32) using a large, nationally representative sample and the state of the art analytic technique. This approach allowed examination of trajectories of substance use from early

adolescence to young adulthood, identifying trajectories that were consistent with those found in the previous literature, and revealing unique trajectories of substance use that have not been found. Secondly, this study is the first to examine gene–gene interaction effects in predicting trajectories of substance use. By considering effects of DRD4 and 5-HTTLPR in combination, this study contributes to the literature by being the first to report that DRD4 and 5-HTTLPR interact in predicting trajectories of substance use over time and providing support for the idea that an imbalance between the dopaminergic and the serotonergic systems may confer vulnerability for substance use. Although replication is needed, these findings speak to the importance of considering DRD4 and 5-HTTLPR in combination in understanding their effects in relation to substance use. Thirdly, building upon previous literature, this study provided further support for the theoretical proposition that genetic and environmental factors function interactively in influencing development over time. Furthermore, findings from this study demonstrated that there are gender differences in genetic and gene–environment interaction effects in relation to substance use, and thus emphasizing the importance of considering gender in studying genetic effects on substance use.

Conclusions

In conclusion, results from this study demonstrate heterogeneity in trajectories of substance use from early adolescence to young adulthood and the importance of both genetic and environmental factors, as well as their interactions in predicting individuals' likelihood of following different trajectories. Findings suggest that genetic effects on trajectories of substance use may vary depending on parenting quality, gender, and the

specific nature of substance use trajectory. Findings also reflect the phenomenon of epistasis (i.e., gene–gene interaction) and suggest the importance of considering multiple genes in combination, particularly genes involving in the dopaminergic and the serotonergic systems, in understanding genetic effects on substance use. It should be noted that mechanisms underlying genetic effects on substance use remain largely unknown, and thus interpretations of findings in this study are largely speculative. Future studies that investigate mechanisms underlying genetic effects, gene–environment interaction effects, as well as gender differences in these effects will be critical to move this field forward in understanding etiology of substance use. One plausible mechanism to investigate may be the genetically influenced brain functioning processes related to substance use, as brain functioning may serve as a neurobiological endophenotype linking genes to substance use (Euser et al., 2012; Iacono et al., 2000).

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APPENDIX A
TABLES AND FIGURES

Table 1. Number of Participants by Wave, Cohort, and Age

	Age																				Total N
	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
Wave																					
I	1120	1690	2213	2673	2706	2514	833	--	--	--	--	--	--	--	--	--	--	--	--	--	13749
II	--	966	1471	1901	2290	2241	1180	292	--	--	--	--	--	--	--	--	--	--	--	--	10341
III	--	--	--	--	--	--	977	1463	1876	2200	2285	2041	675				--	--	--	--	11517
IV	--	--	--	--	--	--	--	--	--	--	--	--	--	1120	1690	2213	2673	2706	2514	833	13749
Cohort																					
1976	--	--	--	--	--	--	833	292	--	--	--	--	675	--	--	--	--	--	--	833	--
1977	--	--	--	--	--	2514	1180	--	--	--	--	2041	--	--	--	--	--	--	2514	--	--
1978	--	--	--	--	2706	2241	--	--	--	--	2285	--	--	--	--	--	--	2706	--	--	--
1979	--	--	--	2673	2290	--	--	--	--	2200	--	--	--	--	--	--	2673	--	--	--	--
1980	--	--	2213	1901	--	--	--	--	1876	--	--	--	--	--	--	2213	--	--	--	--	--
1981	--	1690	1471	--	--	--	--	1463	--	--	--	--	--	--	1690	--	--	--	--	--	--
1982	1120	966	--	--	--	--	977	--	--	--	--	--	--	1120		--	--	--	--	--	--
Total N	1120	2656	3684	4574	4996	4755	2990	1755	1876	2200	2285	2041	675	1120	1690	2213	2673	2706	2514	833	--

Table 2. Frequencies of Genetic Alleles by Race and Gender

Gene	Total (N = 13749)	White (n = 7747)	Hispanic (n = 2198)	Black (n = 2962)	Asian (n = 842)	Male (n = 6417)	Female (n = 7332)
DRD4							
N7R/N7R	8511 (62.6%)	4824 (63%)	1253 (57.7%)	1657 (56.8%)	777 (92.7%)	4046 (63.8%)	4465 (61.6%)
N7R/7R	4425 (32.6%)	2518 (32.9%)	784 (36.1%)	1062 (36.4%)	61 (7.3%)	1992 (31.4%)	2433 (33.6%)
7R/7R	654 (4.8%)	320 (2.4%)	135 (6.2%)	199 (6.8%)	0 (0%)	301 (4.7%)	353 (4.9%)
5-HTTLPR							
LL	4783 (34.8%)	2514 (32.5%)	544 (24.8%)	1635 (55.3%)	90 (10.7%)	2193 (34.2%)	2590 (35.4%)
L/S	6315 (46.0%)	3793 (49.0%)	1064 (48.5%)	1092 (36.9%)	366 (43.6%)	2990 (46.7%)	3325 (45.4%)
S/S	2630 (19.2%)	1429 (18.5%)	585 (26.7%)	232 (7.8%)	384 (45.7%)	1221 (19.1%)	1409 (19.2%)

Note. Values are expressed as number (percentage). 159 participants were missing on DRD4 data. 21 participants were missing 5-HTTLPR data. N7R = no 7-repeat allele, 7R = 7-repeat allele. L = long allele , S = short allele.

Table 3. Model Fit Indices for Growth Mixture Models of Alcohol Use: Males and Females

Number of classes	Male		Female	
	Adjusted BIC	Entropy	Adjusted BIC	Entropy
2	79114.114	.83	81280.401	.85
3	77809.327	.71	79394.073	.79
4	76742.800	.75	78718.650	.78
5	76231.951	.75	78089.163	.76
6	75913.560	.75	77605.191	.77

Table 4. Model Fit Indices for Growth Mixture Models of Cigarette Use

Number of classes	Male		Female		Whole Sample	
	Adjusted BIC	Entropy	Adjusted BIC	Entropy	Adjusted BIC	Entropy
2	57133.826	.97	59268.130	.97	117715.296	.97
3	52616.186	.95	54592.071	.96	108729.673	.95
4	49846.014	.96	50178.235	.97	102057.828	.96
5	48441.342	.96	48437.431	.96	98633.766	.96
6	47093.840	.96	46665.133	.97	95573.673	.96

Table 5. Model Fit Indices for Growth Mixture Models of Marijuana Use

Number of classes	Male		Female		Whole Sample	
	Adjusted BIC	Entropy	Adjusted BIC	Entropy	Adjusted BIC	Entropy
2	62611.602	.993	63377.005	.998	128413.766	.996
3	57898.126	.991	57495.925	.997	118516.886	.991
4	54712.279	.988	52915.302	.996	110630.398	.992
5	51215.611	.994	49708.058	.991	104976.991	.992
6	52170.000	.985	45256.599	.996	99158.836	.993

Table 6. Predicting Alcohol Use Trajectories among Males

	Escalators		Adult-Quitters		Persistent Heavy Drinkers	
	Model I	Model II	Model I	Model II	Model I	Model II
Hispanic	-.47**	-.47**	-.29	-.31	-.62	-.59
Black	-1.44***	-1.45***	-.94***	-.95***	-1.99***	-1.89***
Asian	-1.05**	-1.06**	-1.65**	-1.62**	-1.38	-1.45
Maternal education	.11**	.11**	.02	.02	.01	.01
DRD4	-.02	-.02	-.24	-.23	-.17	-.19
5-HTTLPR	.26*	.29*	.07	.09	.46+	.35
Parenting quality	.09*	.15	-.08*	-.13*	-.23***	-.01
DRD4 X 5-HTTLPR	--	--	--	--	--	--
DRD4 X Parenting	--	--	--	--	--	--
5-HTTLPR X Parenting	--	-.09	--	.09	--	-.30*
DRD4 X 5-HTTLPR X Parenting	--	--	--	--	--	--

Note. A series of multinomial logistic regressions were conducted. Model I presents coefficients from model including only control variables and main effects. Model II presents logit coefficients from the final models examining interaction effects. Experimenters was the reference trajectory. White was the reference category for race. + $p < .10$, * $p < .05$, ** $p < .01$, *** $p < .001$. -- indicates not included in the model. N = 5648.

Table 7. Predicting Alcohol Use Trajectories among Females

	Escalators		Developmentally-Limited Drinkers		Adult-Quitters	
	Model I	Model II	Model I	Model II	Model I	Model II
Hispanic	-.34	-.34	-.11	-.11	-.72	-.76
Black	-2.06***	-2.04***	-1.01***	-1.03***	-2.76***	-2.77***
Asian	-.68*	-.65*	-.54	-.54	-4.18	-213.76***
Maternal education	.13***	.13***	-.05	-.05	.08	.08
DRD4	.09	.08	-.05	-.11	-.11	-1.17
5-HTTLPR	-.04	-.05	-.29+	-.31	.01	-.30
Parenting quality	-.01	-.09	-.23***	-.24**	-.13*	-.05
DRD4 X 5-HTTLPR	--	.01	--	.14	--	1.34
DRD4 X Parenting	--	.23	--	-.05	--	-.42
5-HTTLPR X Parenting	--	.14	--	-.01	--	-.11
DRD4 X 5-HTTLPR X Parenting	--	-.35+	-	.14	--	.53

Note. A series of multinomial logistic regressions were conducted. Model I presents coefficients from model including only control variables and main effects. Model II presents coefficients from the final models including interaction effects.

Experimenters was the reference trajectory. White was the reference category for race. + $p < .10$, * $p < .05$, ** $p < .01$, *** $p < .001$. -- indicates not included in the model. N = 6563

Table 8. Predicting Cigarette Use Trajectories with the Whole Sample

	Escalators		Adult-Quitters		Persistent Heavy Smokers	
	Model I	Model II	Model I	Model II	Model I	Model II
Male	.45***	.37**	-.19	-.28	.21	.23
Hispanic	-1.20***	-1.20***	-1.30***	-1.29***	-1.51***	-1.50***
Black	-.55***	-.54***	-2.21***	-2.21***	-2.84***	-2.85***
Asian	-.52**	-.49**	-1.30***	-1.27***	-2.18***	-2.15***
Maternal education	-.08***	-.08***	-.06*	-.06*	-.21***	-.21***
DRD4	.11	-.07	-.23	-.30	.01	-.14
5-HTTLPR	-.12*	-.30**	.18	.12	-.15	-.19
Parenting quality	-.06**	-.13**	-.25***	-.30***	-.20***	-.22**
DRD4 X 5-HTTLPR	--	.27+	--	.10	--	.23
DRD4 X Parenting	--	--	--	--	--	--
5-HTTLPR X Parenting	--	.11*	--	.07	--	.09
DRD4 X 5-HTTLPR X Parenting	--	--	--	--	--	--
Male x DRD4	--	--	--	--	--	--
Male x 5-HTTLPR	--	.13	--	.12	--	-.07
Male x parenting quality	--	.08	--	.01	--	-.07
Male X DRD4 X 5-HTTLPR	--	--	--	--	--	--
Male X DRD4 X Parenting	--	--	--	--	--	--
Male X 5-HTTLPR X Parenting	--	-.15+	--	-.03	--	-.04
Male X DRD4 X 5-HTTLPR X Parenting	--	--	--	--	--	--

Note. A series of multinomial logistic regressions were conducted. Model I presents coefficients from model including only control variables and main effects. Model II presents coefficients from the final models including interaction effects. Non-smokers/experimenters was the reference trajectory. White was the reference category for race. + $p < .10$, * $p < .05$, ** $p < .01$, *** $p < .001$. -- indicates not included in the model. N = 12,211.

Table 9. Predicting Marijuana Use Trajectories with the Whole Sample

	Early escalators		Late escalators		Quitters	
	Model I	Model II	Model I	Model II	Model I	Model II
Male	.81***	.77***	.39**	.36	.39*	.28
Hispanic	-.27	-.27	-.05	-.05	-.14	-.14
Black	.07	.07	-.19	-.19	-.62**	-.62**
Asian	-.64*	-.65*	-.28	-.28	-1.20*	-1.20*
Maternal education	-.00	-.003	.06*	.06*	-.04	-.04
DRD4	-.08	.22	.13	.09	-.10	-.13
5-HTTLPR	-.11	-.34+	-.11	-.10	-.15	-.22
Parenting quality	-.12***	-.12***	-.16***	-.16***	-.24***	-.24***
DRD4 X 5-HTTLPR	--	--	--	--	--	--
DRD4 X Parenting	--	--	--	--	--	--
5-HTTLPR X Parenting	--	--	--	--	--	--
DRD4 X 5-HTTLPR X Parenting	--	--	--	--	--	--
Male x DRD4	--	-.48**	--	.08	--	.05
Male x 5-HTTLPR	--	.37+	--	-.01	--	.14
Male x parenting quality	--	--	--	--	--	--
Male X DRD4 X 5-HTTLPR	--	--	--	--	--	--
Male X DRD4 X Parenting	--	--	--	--	--	--
Male X 5-HTTLPR X Parenting	--	--	--	--	--	--
Male X DRD4 X 5-HTTLPR X Parenting	--	--	--	--	--	--

Note. A series of multinomial logistic regressions were conducted. Model I presents coefficients from model including only control variables and main effects. Model II presents coefficients from the final models including interaction effects. Non-users/experimenters was the reference trajectory. White was the reference category for race. + $p < .10$, * $p < .05$, ** $p < .01$, *** $p < .001$. -- indicates not included in the model. N = 12,210

Figure 1. Alcohol Use Trajectories Among Males

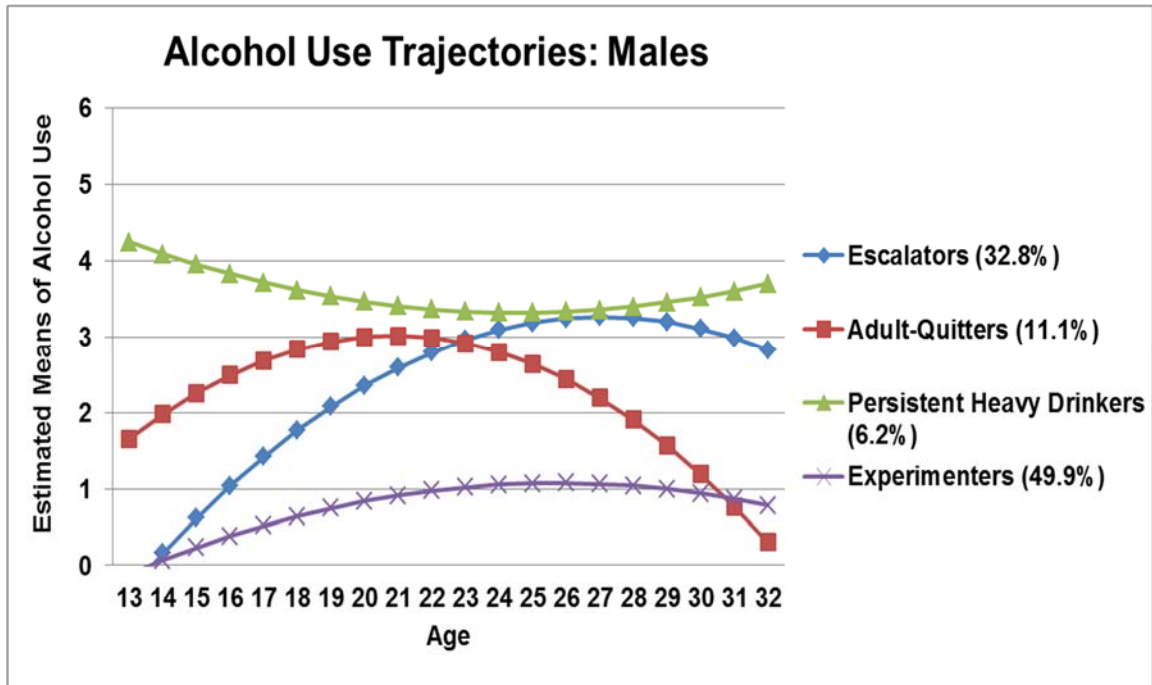


Figure 2. Alcohol Use Trajectories Among Females

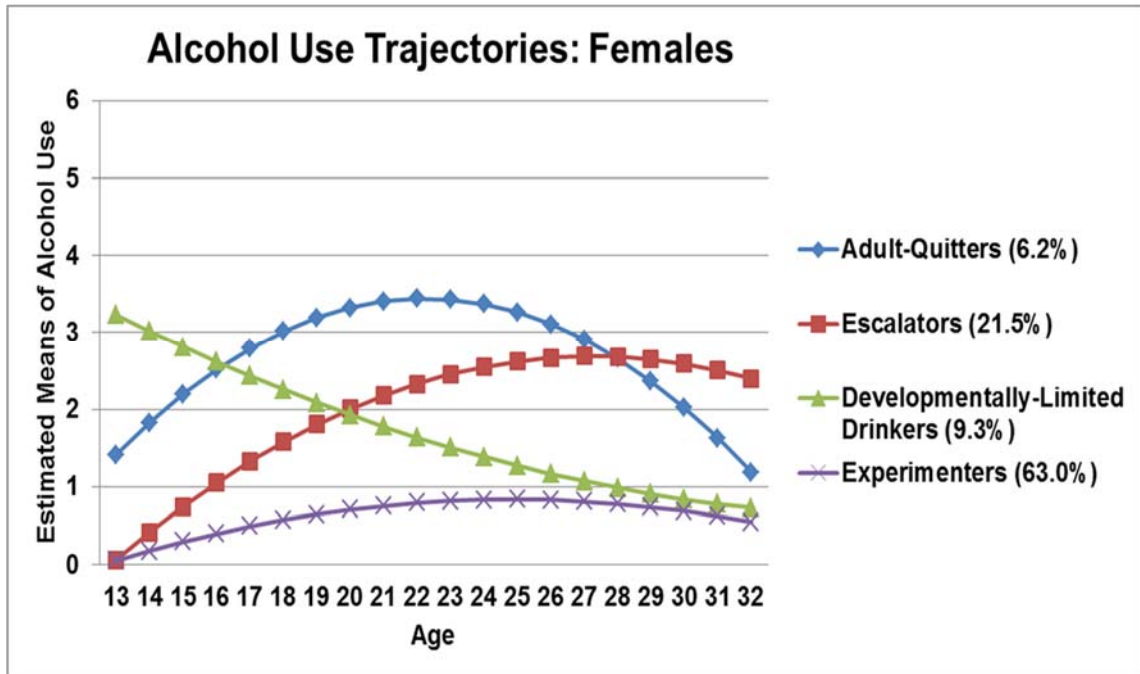


Figure 3. Cigarette Use Trajectories: Whole Sample

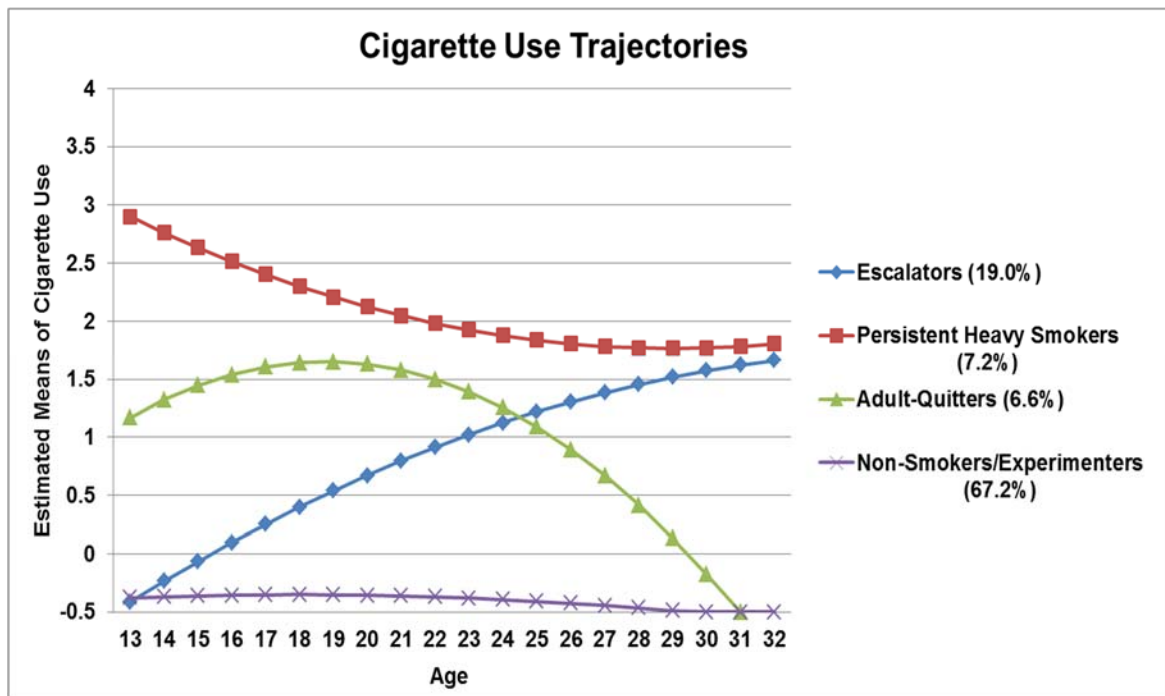


Figure 4. Marijuana Use Trajectories: Whole Sample

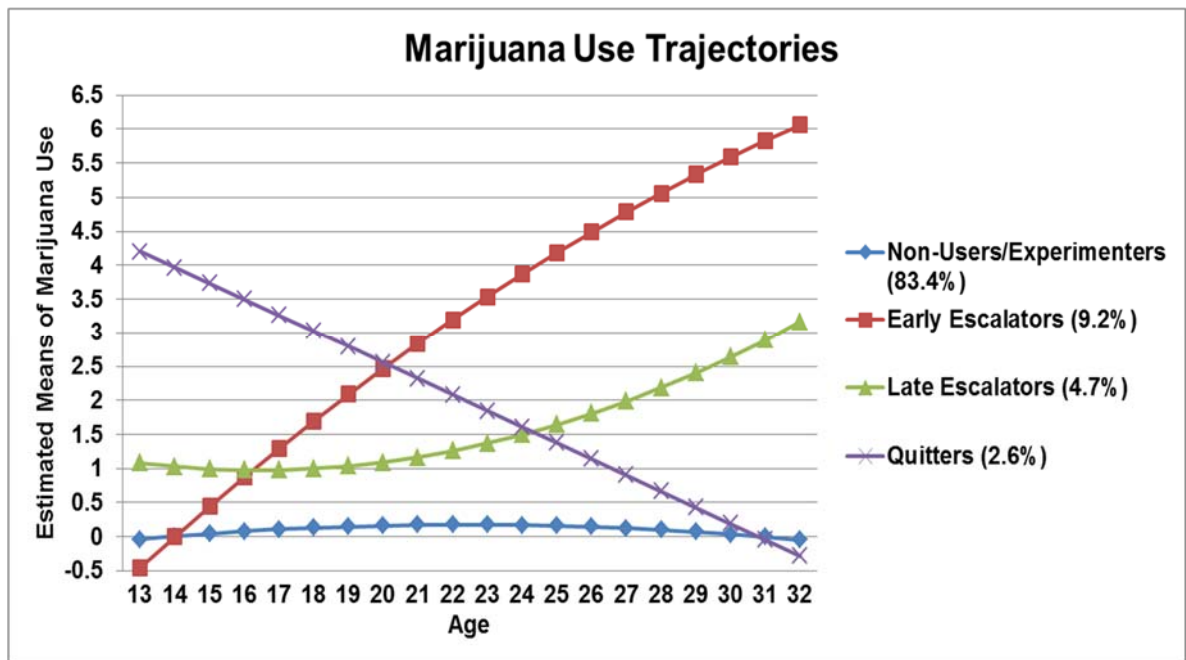


Figure 5. Interaction between Parenting Quality and 5-HTTLPR in Predicting Likelihood of Following the Persistent Heavy Drinkers Trajectory Among Males

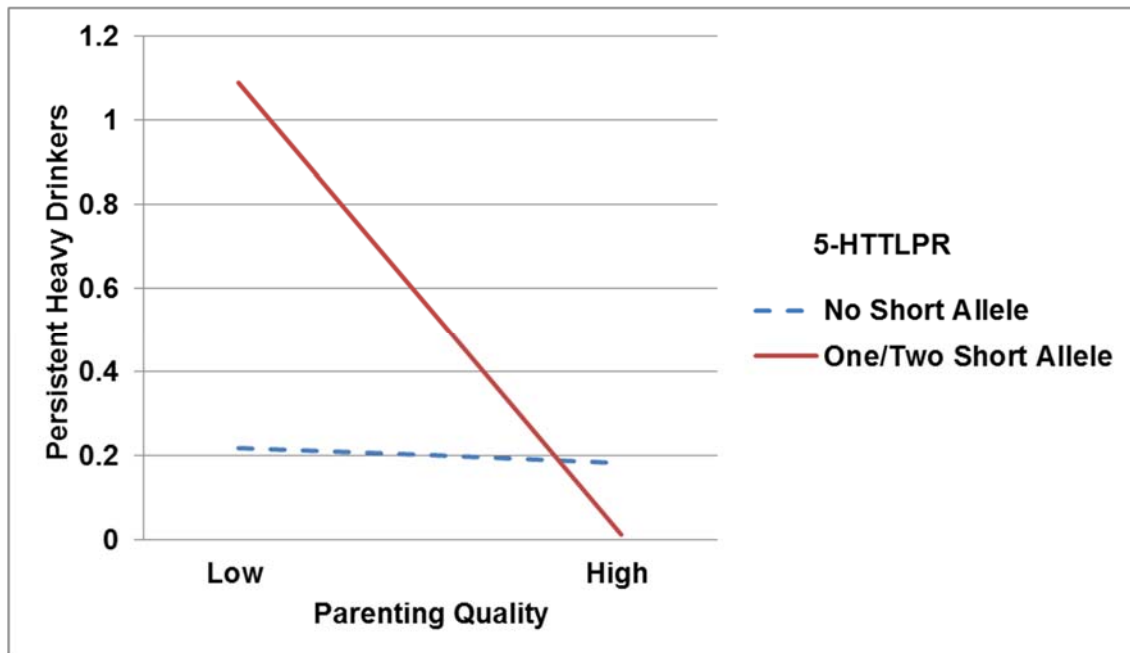
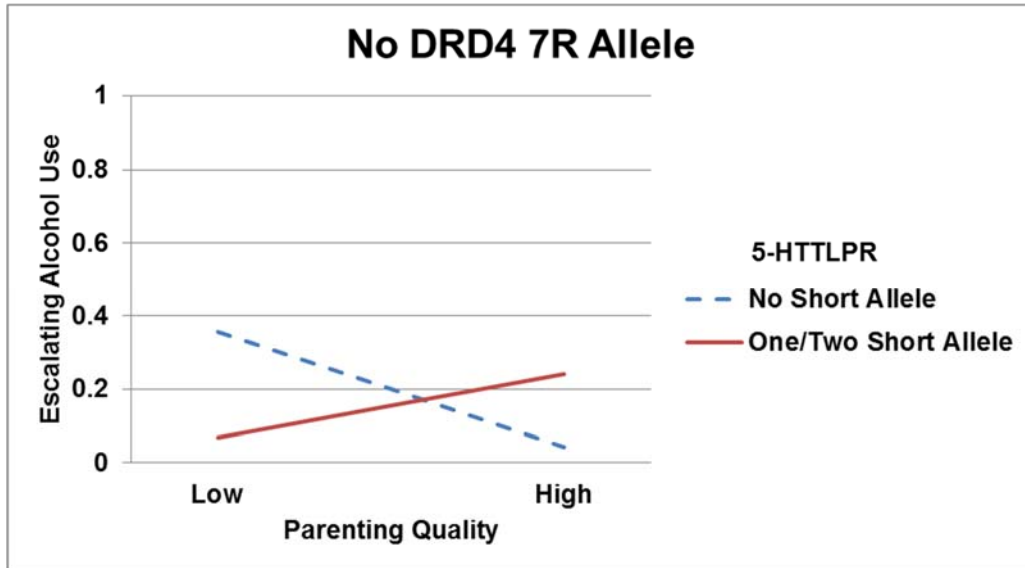


Figure 6. Parenting Quality by 5-HTTLPR by DRD4 Interactions Predicting Likelihood of Following the Escalators Trajectory of Alcohol Use among Females

Panel A.



Panel B.

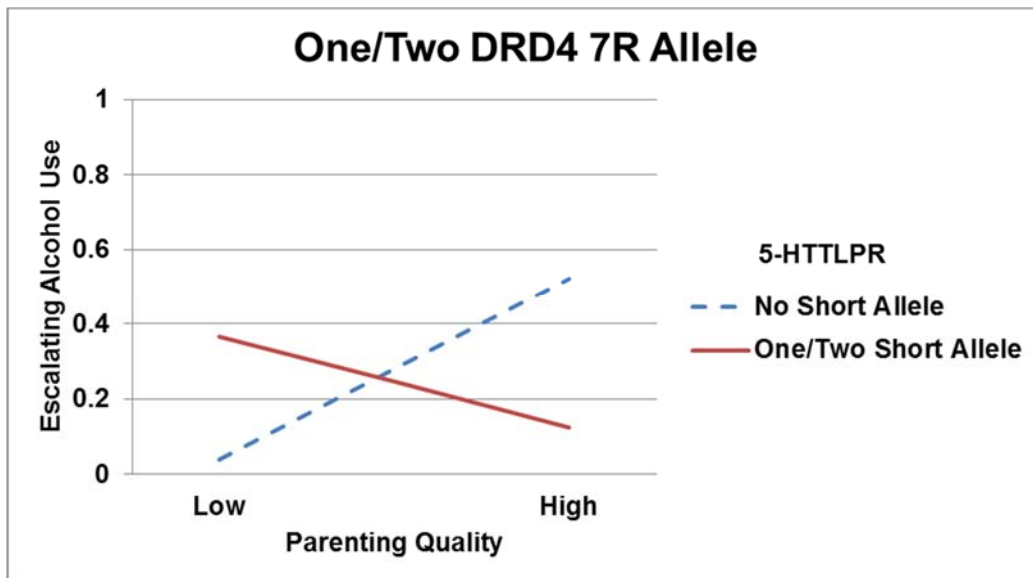


Figure 7. DRD4 by 5-HTTLPR Interaction Predicting Likelihood of Following the Escalators Trajectory of Cigarette Use

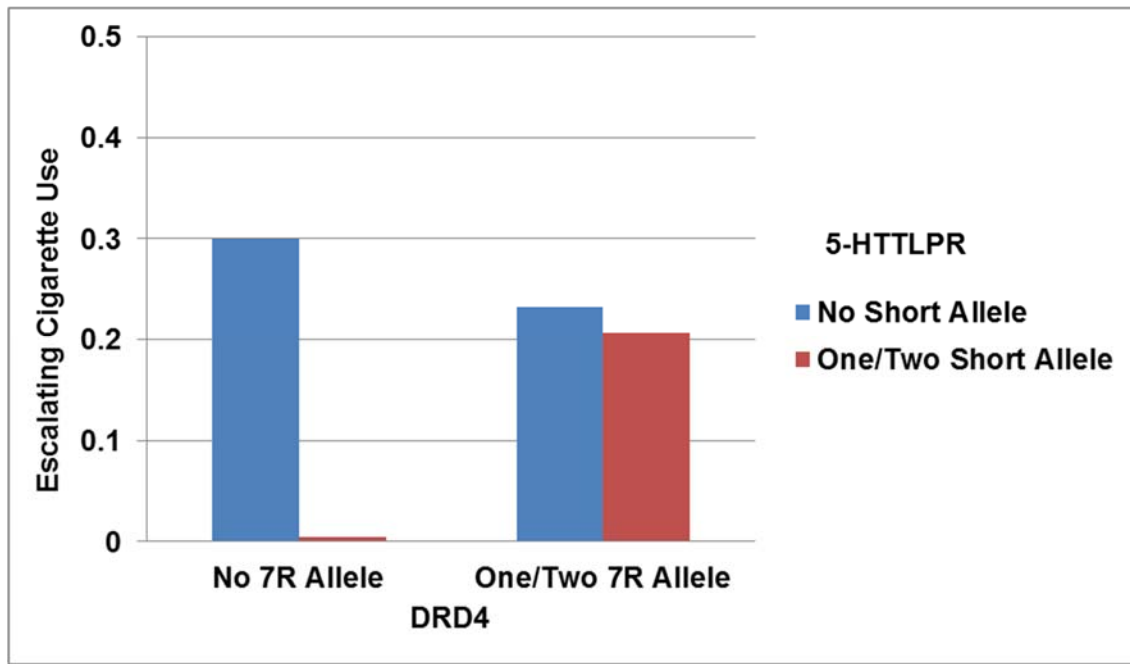
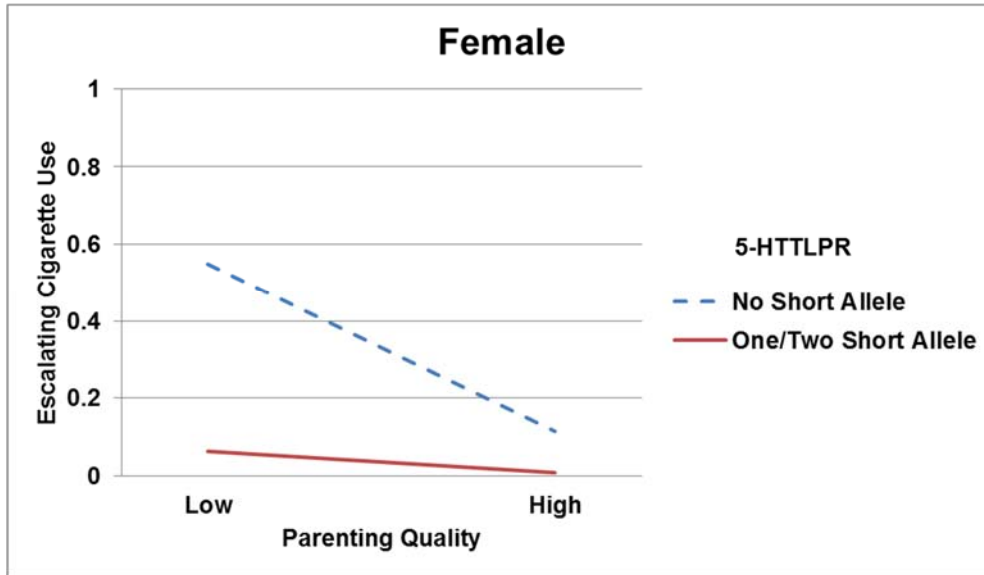


Figure 8. Parenting by 5-HTTLPR by Gender Interaction Predicting Likelihood of Following the Escalators Trajectory of Cigarette Use

Panel A.



Panel B.

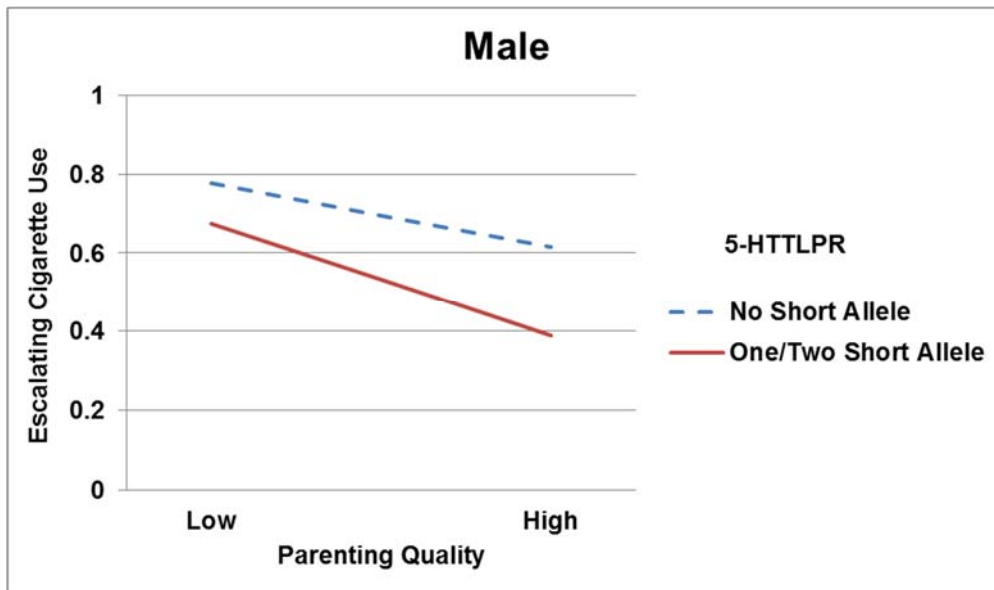


Figure 9. DRD4 by Gender Interaction Predicting Likelihood of Following the Early-Escalators Trajectory of Marijuana Use

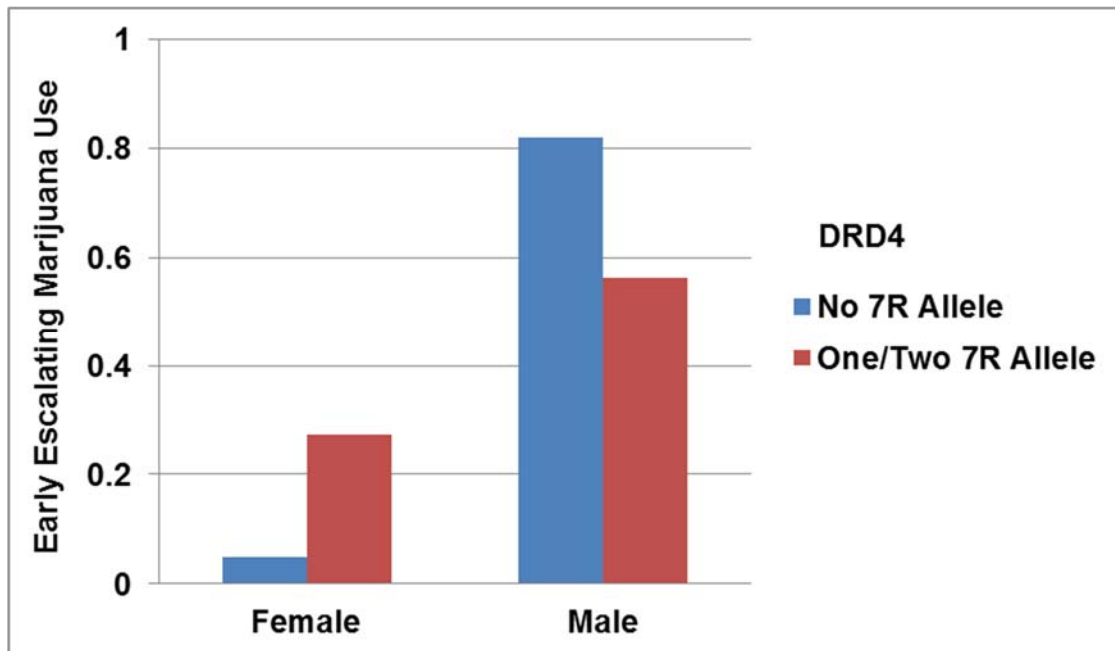


Figure 10. 5-HTTLPR by Gender Interaction Predicting Likelihood of Following the Early-Escalators Trajectory of Marijuana Use

